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TO VESALIUS ON THE FOURTH CENTENARY OF HIS *DE HUMANI CORPORIS FABRICA*

By CHARLES SINGER

The month of June 1943 marks the fourth centenary of an event memorable for the anatomist; but it does more, it marks a period for the history of science, and indeed for the whole of modern civilization. In June 1543 there appeared at Basel, from the press of Johannes Oporinus (Johan Herbst, 1507–68), the *De humani corporis fabrica* of Andreas Vesalius (1514–64). It is the earliest book to present systematically new knowledge won by observation in what is immediately recognizable as the manner of our own day. It is, in fact, the first great work of modern science.

The time was noteworthy for its scientific output. Thus only a few weeks earlier appeared the *De revolutionibus orbium celestium* of the Pole, Nicolas Copernicus (1473–1543), who died as it was going through the press. A few weeks before that, Leonhard Fuchs (1501–66) issued at Basel his great *Historia stirpium*, most beautiful and famous of herbals. Neither is at all comparable to the great work of Vesalius. In the following century revolutionary ideas were advanced in the name of Copernicus, but, of a truth, his book is in no way modern. It is conservative in method, scholastic in tone, and hardly at all based on observation, though, as things transpired, it was the starting point for modern astronomy. Fuchs, an accurate, scholarly and industrious observer, set a standard for plant illustration which, perhaps, has not since been passed, but he exhibits no trace of that great creative drive distinctive of scientific genius. Indeed, in the whole of his own century Vesalius had only two peers with whom his great spirit could have joined in equal converse. One was Leonardo da Vinci (1452–1519), who died when Vesalius was five; the other was his own countryman, Simon Stevin of Bruges (1548–1620), who was not sixteen when Vesalius ended his life on a Greek island. Even these men do not measure by Vesalius either in completeness of achievement or in influence on the generations that followed. In his own century Vesalius walks alone. It is a fair statement that the modern period of science opens with his great book.

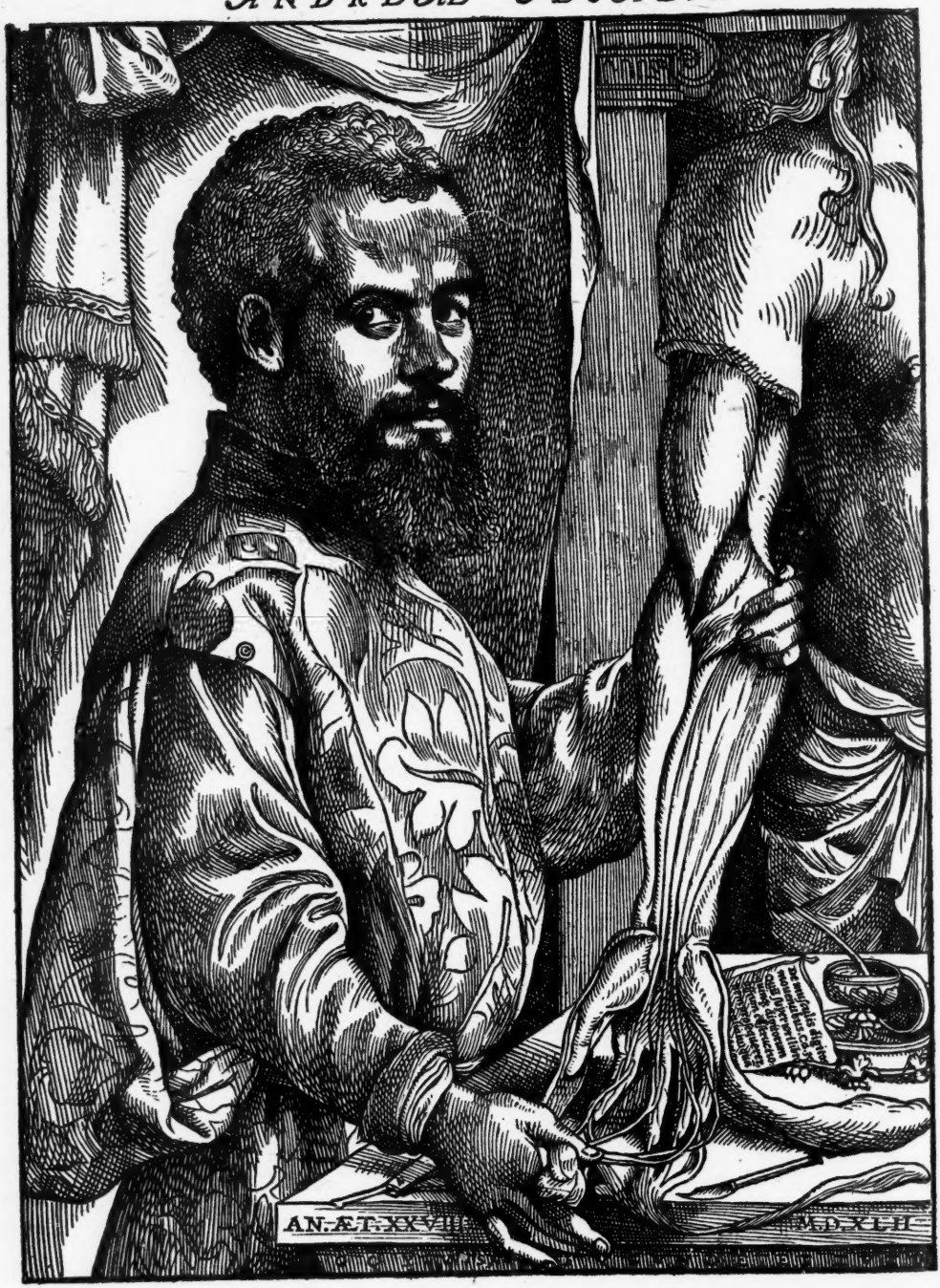
André Vésale—that was the real form of his name—was born at Brussels in the night between 31 December 1514 and 1 January. His father was Apothecary to the Emperor Charles V to whom Andreas was later Body-physician. His mother was perhaps of English extraction, for her maiden name was Isabel Crabbe. He says himself that, as a lad, he was constantly dissecting bodies of animals. Such tastes are common enough with boys nowadays. Nature study is taught in schools and it is easy to get help from books. But the study of nature was then little regarded and there were as yet no such books. Vesalius had to find his own way.

He studied at the Universities of Louvain and Paris. Both were strongholds of academic conservatism. At both the teaching of anatomy was quite medieval in manner and substance. The professor, when lecturing, mounted into his *Chair*, a great elevated structure like a pulpit and provided with a reading desk. Thence he read from the text of Galen—hence the academic title *Reader*—commenting on it as he went, while a junior colleague, the *Ostensor*, indicated the line of incision and a menial *Demonstrator* did the actual dissection. All was thus done third hand and according to the written word. By 1537 Vesalius had quarrelled with his teachers and their methods, had shaken the dust of Paris from his feet, and had made for the land of humanistic light, Italy. There in 1538 he issued the first of his works that shows traces of the scientific spirit. It is in six sheets and known as the *Tabulae anatomicae*.

The six tables are of very large folio size and were drawn by a pupil of the artist Titian, Jan Stephen van Calcar (1499–1546), who arrived in Italy about the same time as Vesalius. These splendid diagrams show many details that can have been learned only by careful dissection, but the spirit of Galen still broods over them. Thus, for example, they still ascribe to the human body the five-lobed liver of the pig, the rete mirabile of the calf, the long hepatic vein and the truncus brachiocephalicus of the dog, the long, protruding coccyx of the ape, the sternum in seven segments of an ungulate, together with the venous system arising from the liver of Galen's imagination.

While these *Tabulae* were in the press Vesalius was appointed professor of 'the surgical part of medicine' at the University of Padua. He protests, in the preface of his great book, that medicine can be effective only if firmly based on anatomy, and that to this subject he had therefore resolved to give his main attention. To make anatomy the positive basis of medicine was itself a very great reform, but he did more, for he changed completely the whole content and tradition of anatomy itself. His fundamental reform was to do away with 'demonstrators' and 'ostensors' and to put his own hand to the business of dissection. He was soon gathering large audiences. His lectures were given on the human body itself, displayed with his own

ANDREAE VESALII.



hands and with living models, drawings and skeletons at hand. Animals, too, were available for experiment and to illustrate special points. There can be no doubt that, not infrequently, he had to content himself with animals rather than the human subject for many anatomical details.

Vesalius was now master in his own department. His great research was carried out with an almost fierce energy. Every line and every figure of his epoch-making book, the product of but five years' activity, is instinct with his virile power. He was still but 28 when it was issued at Basel, almost simultaneously with a companion *Epitome* intended rather for artists than anatomists. With this his life work was completed. A second edition appeared in 1555 and contains definite improvements but no fundamental changes. From 1543 onward anatomy becomes Vesalian, while Vesalius himself passes into the background. We shall not follow his life further for it is not of first importance for the history of anatomy. For that, Vesalius and his great treatise are one. Without the book he would be but a ghost.

It may be pointed out that the name of Vesalius has hardly any place in anatomical nomenclature. In this he differs from many great anatomists. It is just that it should be so, for he has impressed his personality on the whole *fabrica*. That word needs some elucidation. It must by no means be translated 'fabric', and even 'mechanism' hardly renders it. In its ancient classical usage it means 'an artisan's workshop', essentially a place where something is going on and, by transference, the art or trade itself. This meaning is reflected in modern German *Fabrik*, factory, but better in modern French *fabrique* which means both the process of making and the place where things are made. In Renaissance Latin too the word has a kinetic meaning. I think a good—if unliterary—rendering would be 'works' or 'workings'. *De humani corporis fabrica*, 'On man's bodily workings'. Remember the book was both physiological and anatomical, for Vesalius was always trying to describe living anatomy. I can believe, therefore, that anatomists may still have something to learn from his figures, for he had an artist's eye, if ever a man had.

Moreover, not only was Vesalius a student of living anatomy but, as a corollary, it was always the body as a whole that was in his mind. This vision of the living *fabrica* made him a great creative anatomist, but it made him, at the same time, a great creative artist. Yet this vision makes his work difficult reading, for to understand it we must rid ourselves of certain ideas that come to us nowadays almost instinctively. To understand him we must think as renaissance artists and not as modern evolutionists.

The modern scientific biologist deals primarily with description and secondarily with origin. He takes an organ or part or function or reaction or group of organisms for its own sake and investigates it in detail. Then he treats it comparatively and then ontogenetically and then phylogenetically. His thought is always saturated with evolutionary implications. Nor is this the case only since Darwin, for evolution was implicit in biological thought long before him. But with Vesalius it is not so. For him the body is a *fabrica*, a piece of living workmanship, made by the Great Craftsman. The parts must be considered always in relation to the scheme as a whole, must be treated as contributing to that complex that we call a man. Thus, for example, his great general figures of muscles or of bones are not diagrammatically displayed from front, back, and side, but posed as in the living body. They are given tone and movement and provided with a background such as that which they have in life. Again in the figure of the vertebral column, the bones are not displayed in diagrammatic succession but an attempt is made to arrange them in the curves that they present in the living subject. This method is used with all the artist's skill and was his own. We need not discuss the share that Calcar or any other artist had in the actual drafts. For our purpose this is irrelevant, since the artist's mind that conceived them was clearly that of Vesalius.

Moreover, Vesalius, child of his age, could not, even as anatomist, help thinking of the end for which man was made. If he was deeply influenced by Renaissance Art he was also the product of the Revival of Learning. He was steeped in Galen, nor could he shake off Galenic teleology even if he would. But, with an artist's mind and eye, Vesalius transformed that moss-grown scheme into something characteristic of his time and his genius. For him man is a work of art, God an artist. He had in him little of the philosopher, the theologian or the poet, nor must we seek in his pages for any skilled or formal justification of his teleology. But so much he says, and says well, over and over again. Men and women he saw as the Great Artist's studies for His grand design of His own image. Imperfect studies, indeed. Vesalius, unlike Galen, did not harp constantly on the perfection of man's form. He had before him bodies of criminals, worn-out paupers and those wasted by disease, yet shadowing a unique conception in the mind of the Godhead. It was for the anatomist to reach closer than those poor corpses to the type of perfection. We think of anatomy in terms of evolution, and our questions are always 'Whence?' and 'How?' Vesalius thought in terms of design, and his questions were 'Whither?' and 'Why?'

The vigorous, teeming mind of Vesalius presented another and less attractive aspect. He was very learned, well acquainted with the new-found wealth of antiquity which the Humanists were making more accessible to the reading public. There was no

element of modesty in his psychological composition. He makes a tremendous display of his erudition, and it is sometimes slightly spurious. His books are full of Arabic and Hebrew words. To these he was helped by other scholars, and of those languages his knowledge was, in fact, of the slightest. But we must remember the age in which he lived. The great intellectual battle between 'Arabists' and 'Humanists' was still fierce. The issue was still far from clear. Thus the constant reference to Arabic and Hebrew was something more than mere vain show, though it must be allowed there is much of the showman in Vesalius. With the Greek language, however, he had considerable facility. So far as his reading in the available Latin translations of the Greek, Arabic, and Hebrew classics was concerned, there can be no doubt that he was highly accomplished and more critical than most in his time.

The anti-Galenism of Vesalius has been overestimated both by himself and by others. In the very years in which he was most busily occupied on the *Fabrica* his leisure was largely devoted to editing Galenic works. The Galenism of his *Tabulae anatomicae* (1538) certainly represents a comparatively early stage in his development, but not so his editions of works of Galen himself. For the great edition of Galen, produced by the printing firm of Giunta at Venice in 1541 and the following years, Vesalius edited the works *On the dissection of the nerves*, *On the dissection of the veins and arteries*, and the very important treatise *On anatomical procedure*. Moreover, the numberless references to Galen in the *Fabrica* exhibit his respect for his greatest predecessor who died twelve hundred years before he was born. Indeed, it was hardly open to him to do other than build on Galen. The Galenic works, accessible to him in good modern versions, presented by far the best current anatomical accounts. The tone of much of his criticism of Galen is not that which a modern writer would be allowed to adopt, but it was well within the taste of the time. It is thus not remarkable that many Galenic anatomical terms entered the anatomical vocabulary of Vesalius. Many more he borrowed from contemporary humanist physicians, but some he invented for himself, among them, for example, *Atlas* (as applied to the first cervical vertebra), *Alveolus*, *Choanae* (in the modern connotation), *Corpus callosum*, *Incus*, and *Mitral Valve*.

We must, then, think of Vesalius as trebly equipped for his task; first, by a wonderful native genius for anatomical observation and classification; secondly, by great powers of imagination, exalted by contact with high models of Renaissance Art; thirdly, by an admirable education, according to the standards of the time, directed along humanist lines by some of the ablest medical humanists of the day. But we must also think of him as most happily constituted for his task by the *lack* of certain qualities which would have handicapped him in that age. He was not interested in philosophical or theological problems. He was not at all given to the drawing of those vague physical analogies characteristic of the men of his time who borrowed many such ideas from Aristotle. He was saved from diversion into the miraculous or magical by a mind full of what he actually saw and did. He was a thorough extrovert of stable, unsubtle, restless, constructive mind with an artist's eyes, hands and imagination, the very type and exemplar of the modern man of science.

The portrait of Vesalius which first appeared in his *Fabrica* has attracted an immense amount of attention and no little undeserved adulation. In putting it again before the reader I ask him to approach it as though he was seeing it for the first time, for I have endeavoured to approach it myself in that fashion.

It is obviously a 'speaking' likeness of a real man. The great head seems full of energy. Eyes and mouth suggest quick wit and ready humour. It is the facies of a man of action, and it is easy to feel that this is one who could construct the great book. But, apart from the head, the drawing seems to me execrable. The perspective is wrong throughout. The relative proportions of hand, forearm, upper arm, body, and head are impossible. The right hand is without a wrist. The body that he is dissecting is that of a giantess of some seven feet or more and her hand is three times the size of Vesalius's own. Even the rendering of the scroll on the table contains childish blunders.

I am quite unable to believe that this picture was composed by Calcar or by any competent or adequately trained artist. No pupil of Titian could possibly have so botched his anatomy. The history of the picture seems to me to be quite plainly written on it. Some good artist—Calcar or another—drew or painted a *head* of Vesalius. This was recognized as a success and it was placed in the hands of a woodcutter, who received directions from Vesalius as to the setting in which it was to be placed. The craftsman copied the head slavishly, line for line and point for point, and finished by putting it in a composition of his own, which is obviously not the work of a trained artist. Vesalius himself must have provided him with a drawing of the anatomical details. (That Vesalius was a good draftsman we know on other grounds.) Vesalius was pleased with the picture as a whole for he reproduces it several times. The reason for his satisfaction was, I am sure, the extremely happy treatment of the head.

There remain a few details to elucidate. On the top edge of the column or table is inscribed the phrase AN[NO] AET[ATIS] XXVIII—MDXLII so that the figure was engraved the year before the publica-

ANDREAE VESALII
BRVXELLENSIS, SCHOLAE
medicorum Patauinæ professoris, de
Humani corporis fabrica
Libri septem.

BASILEAE.

UNIV. OF MICH.

tion of the book and in his 28th year. Below is indistinctly inscribed OCYUS IUCUNDE ET TUTO. This I take to be a memory from Celsus who quotes Asclepiades as saying that treatment should be *tuto, celeriter, jucunde* 'safe, swift, seemly'. The writing on the scroll remains a puzzle. It is an inaccurate transcript of the first sentence of Book I, Chapter 43 (*not* chapter 30 as stated), p. 304 of the great treatise. The character of the errors suggests that the woodcutter knew no Latin and perhaps could barely read. The passage in the treatise corrects Galen's view of the flexor apparatus of the hand. Its insertion in the picture is perhaps intended to indicate opposition to him. Galen was particularly interested in the hand and wrote on its structure in great detail, drawing his knowledge of it from the hand of the Barbary ape. Vesalius specially records his improvement on Galen's treatment of the subject in the Preface to the *Fabrica* (p. 4).

The Frontispiece of the *Fabrica* is in a very different position to the portrait. It was in all probability from a design of Calcar. Like the portrait it is a woodcut, not a copperplate. It is ranked by connoisseurs very high in its class alike in conception, arrangement, character, drawing, 'colour' and cutting. Much symbolism has been discovered in it and all or nearly all, in my opinion, unnecessary, misplaced, and mistaken. Vesalius was not at all given that way. He had a taste for satire, caricature and whimsy—witness his very entertaining historiated initials,—but symbolism—No!

It is a very crowded and animated scene. Vesalius stands in the centre of a circular domed building. On the table before him is a partly dissected female body and an articulated skeleton. He demonstrates to an assembly of some eighty persons, placed in three tiers, separated from each other and from the central group around him by a series of three bars.

In the foreground is a group of menials. Two sharpen instruments, one holds a panting dog and another a chained monkey which a student is teasing. There is no reason to give any symbolical meaning to these animals for Vesalius used their anatomy to illustrate his book. Around him are gathered persons of importance. On his left a bearded sage gazes intently at the dissection. The old man on his right pulls his writing tablet from his pouch. On the opposite side of the table another patriarch motions the servants to silence. Third to the right of this tactician is a figure wearing spectacles which had become quite common at that date.

Behind the first two bars are crowded younger and mostly hatless students. Their faces indicate close attention. To the extreme right is a listener in monastic garb and in front of him a bearded student holds a large eyeglass. On the lateral pilasters are two figures with which the symbolists have greatly disported themselves. That to the left is a thin elderly man whose muscular outlines are conspicuous. I suggest that he is a model used for surface markings. His opposite number on the right is a young rake wearing dandified slashed hose. Hat over eyes, he is giving what seems to me a tipsy lurch. He is, I believe, up to some mischievous prank of which the monk in front of him tries to look unconscious.

The hindermost tier is perhaps the most interesting. The figures here represent the lay public and those of other faculties, some in outdoor dress, drawn to listen to this ornament of the university. What will he say next? Some are arguing together. Most are less attentive than the students in front. One pernicketty young scholar has brought a volume of Galen in which he and another follow the text closely. They will certainly detect divergence from it during the demonstration. To the right of these two stands another solemn, not to say somnolent, monk. Between the pillars at the back a few ladies may perhaps be seen. Heads peep through the clerestory windows at the top. Between them two putti support an heraldic shield on which are three weasels, the emblem of Vesalius.

Does this picture represent a real building and was there ever an anatomical theatre at all like this at Padua? It has been much discussed. In my opinion the answer is a definite negative. There is no record of any special anatomical theatre at Padua till one was built in 1583 when Vesalius had long been dead. I believe that all these architectural features are drawn from the always expansive imagination of Vesalius. We do not know where he did most of his dissection, but I think I can make a fair guess as to the place that he had in mind for this scene. In the quadrangle that makes up the old university buildings at Padua there is, in the wall opposite the entrance, a large apse. This is a natural meeting place for discussion and its form and setting is reminiscent of this imaginary Temple of Anatomy. I believe that is what he has in mind and I believe that what is drawn here is a hope and not a fact. The apse, much more modestly rendered, appears as the scene of an anatomical demonstration in the *De re anatomica* (1559) of the successor to his chair, Realdo Colombo (1516–59), a man of little talent or imagination. Had the Anatomical Temple of Vesalius been a thing of stone and mortar, records and traces of it would certainly have come down to us. But this great book is a more indestructible and more permanent memorial than any building could ever be. On one of the most beautiful and famous of the anatomical figures in it, he has had inscribed

VIVITUR INGENIO: CETERA MORTIS ERUNT

Whatever in him was unreality or error may perish, but his genius is, for all time, alive in this book.

THE HISTOGENESIS OF THE ARTERIES OF THE CHICK EMBRYO

By A. F. W. HUGHES, *Sir Halley Stewart Research Fellow, Strangeways Research Laboratory, Cambridge*

INTRODUCTION

This paper gives an account of the histogenesis of the main arteries of the chick embryo from the time when their structure develops beyond the stage of the simple endothelial wall to the end of the incubation period. It forms part of a wider inquiry into the development of the circulatory system of the chick embryo in which its physiology and structure are being studied side by side.

THE MORPHOLOGY OF THE AORTIC ARCH SYSTEM

In an earlier paper (Hughes, 1934) the main features in the backward migration of the aortic arches in the chick embryo have been described. It was shown that the morphogenesis of the main carotid branches, the subclavian, vertebral and external carotid arteries, is intimately bound up with the migration of the aortic arch system. The previous paper was concerned only with the development of the arteries of the anterior region of the body, all of which are supplied from the carotid arch. Certain features in the morphogenesis of the systemic and pulmonary arches at the period of backward migration remain to be described.

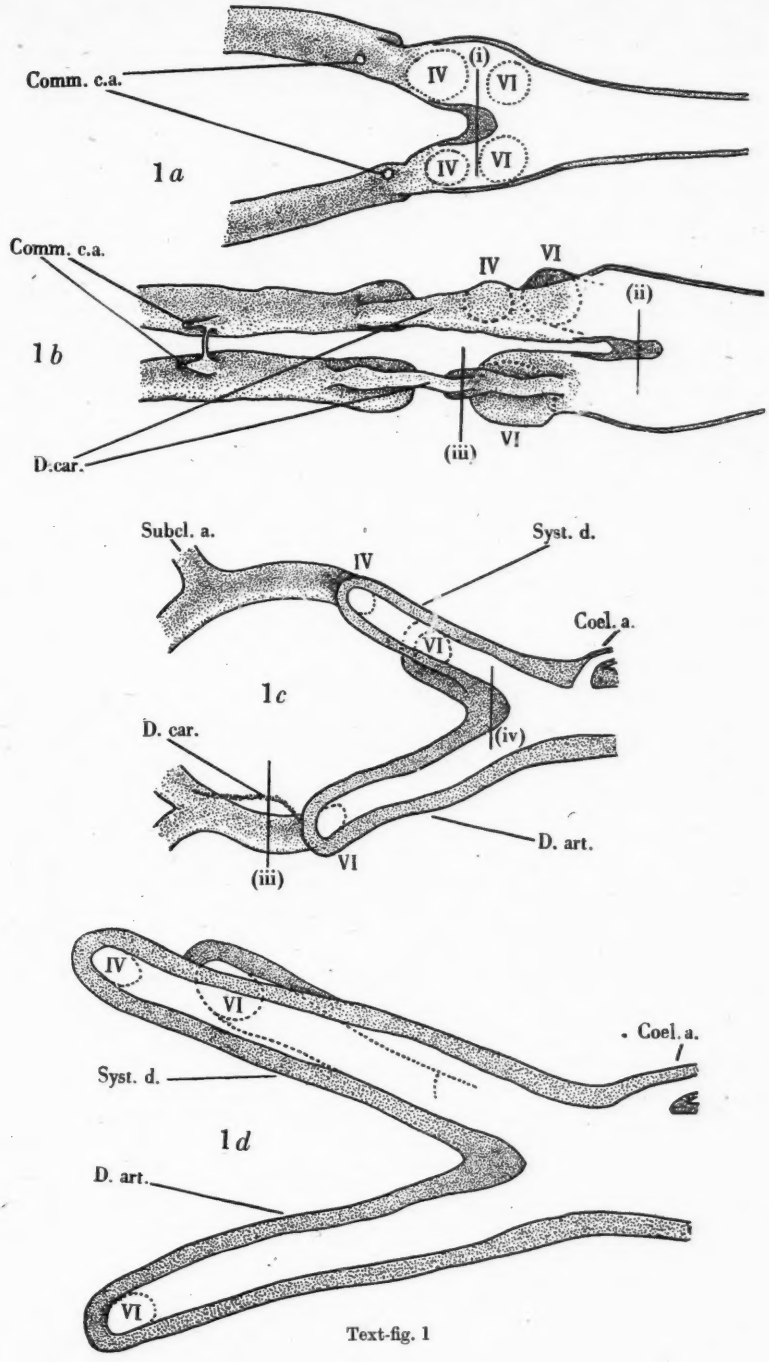
From the primitive aortae, the first-formed intra-embryonic vessels, are derived both the common carotid arteries and the median dorsal aorta. The paired aortae fuse together behind the aortic arches, but not in front of them. At the time of the appearance of the last pair of aortic arches, namely, the sixth, the paired aortae are fused as far forwards as the point of entry of the sixth arches. This point, where the dorsal aorta bifurcates, remains constant until the approach of the fifth day, when backward migration of the arches begins. In Text-fig. 1 the dorsal vessels are reconstructed from section series of embryos from 5 to 9 days of incubation, and in these reconstructions the shift in the point of bifurcation of the aorta can be followed.

As the arches migrate backwards, the carotids become longer, while the median aorta behind the arches becomes shorter. The shortening of the median aorta is accelerated by an additional backward shift of the point at which it bifurcates relative to the aortic arches, which, instead of remaining opposite the sixth arches, is found progressively posterior to them (Text-figs. 1 *b*, *c*). Thus part of the aorta behind the arches undergoes a secondary return to the paired condition. Ultimately, towards the end of the period of backward migration, the point of bifurcation of the aorta is just in front of the origin of the coeliac artery, while previously a long stretch of median aorta extended between the aortic arches and the coeliac artery.

While backward migration is in progress, the shape of the junction between aortic arch and dorsal aorta changes from a T-shaped union at right angles to a form in which the aortic arch bends over, and points either forwards, as in the carotid arch, or backwards, as in the systemic and pulmonary arches, before uniting with the dorsal aorta. The effect of this is that the aortic arches run into the dorsal aortae at an angle so acute that the arch runs almost parallel with the dorsal aorta for some distance before uniting with it. The section of dorsal aorta between carotid and pulmonary arches is very much lengthened, but as it lengthens it decreases in diameter. On the left side it disappears, together with the whole of the left fourth aortic arch, but on the right side, where the systemic arch persists,

Legend to Text-fig. 1

Text-fig. 1. Reconstruction of the dorsal vessels, the common carotid arteries and the dorsal aorta, from embryos of from 5½ to 9 days, to show the point of aortic bifurcation at each stage. Carotids shown in dorsal view; dorsal aorta reconstructed in section. *comm.c.a.* common cervical artery. IV, VI, site of entry of systemic and pulmonary arches respectively into dorsal vessels. *d.car.* ductus caroticus. *syst.d.* systemic ductus. *d.art.* ductus arteriosus. *coel.a.* coeliac artery. *subcl.a.* subclavian artery. (*a*) 5½ days. The aortic bifurcation lies between the systemic and pulmonary arches. Plane of section (i) is that of Pl. 1, fig. 1. (*b*) 6½ days. The aortic bifurcation is now behind the pulmonary arches. Planes (ii) and (iii) refer respectively to figs. 2 and 7 of Pl. 1. (*c*) 7½ days. The aortic bifurcation is well behind the pulmonary arches and is now near the origin of the coeliac artery owing to the shortening of the median dorsal aorta. Planes (iii) and (iv) refer respectively to figs. 4 and 8 of Pl. 1. The carotid arches, seen in surface view, lie at a much lower plane. (*d*) 9 days. This represents the condition found until the end of incubation. Between the aortic bifurcation and the aortic arches is seen on the left side the systemic duct above and the right ductus arteriosus below.


Comm. c.a.
IV
(i)
VI
1a
IV
VI
Comm. c.a.
IV
VI
(ii)
1b
D.car.
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VI
Subcl. a.
Syst. d.
IV
Coel. a.
VI
1c
D. car.
(iv)
D. art.
VI
(iii)
IV
VI
Coel. a.
Syst. d.
1d
D. art.
VI

Text-fig. 1

only the section of dorsal aorta between carotid and systemic (ductus caroticus) degenerates. The systemic and pulmonary arches turn posteriorly at their dorsal ends and enter the dorsal vessel substantially at the same point. At this point the dorsal vessel divides into a dorsal and a ventral half, which continue the right systemic and right pulmonary arches respectively. The dorsal vessel on the right side which is now dividing, however, is itself one-half of the division of the original median aorta. Hence the final result of all these changes is to split the median aorta into three divisions, one on the left side which continues the left pulmonary arch, and constitutes the left ductus arteriosus, and two on the right side. Of these, the lower one is the right ductus arteriosus, and the upper one, at present unnamed, continues the right systemic arch to the median aorta. I propose to refer to this vessel as the 'systemic duct'. These changes can be followed from the dorsal aspect in Text-fig. 1, which should be compared with certain of the lateral reconstructions given in the previous paper (Hughes, 1934, pl. 4, fig. 8; pl. 7, fig. 14; and pl. 12, fig. 20).

Although the ductus arteriosus on either side and the systemic duct originate by the secondary division of the median dorsal aorta, yet the proximal ends of these vessels are formed by dorsal extension of their respective aortic arches. It is not possible in any one of them to determine the boundary between the regions derived from aortic arch and dorsal aorta. In the carotid arch, the dorsal region of the arch turns forwards to such an extent that most of the arch comes to lie nearly parallel with the spinal cord. The root of the vertebral artery, at one time the root of the common cervical artery, marks the original upper limit of the carotid arch (Hughes, 1934, pl. 4, fig. 8).

The rounding of the bend between aortic arch and dorsal aorta has been explained by Kranichfeld on haemodynamic grounds (Kranichfeld, 1914). He draws attention to the fact that the fluid friction generated by flow through a sharp right-angled bend is much greater than that arising from a bend through the same angle at a much greater radius. This explanation is to be placed beside Bremer's demonstration of the haemodynamic cause for the atrophy of the left fourth aortic arch (Bremer, 1928).

EARLY STAGES IN THE DEVELOPMENT OF ARTERIAL WALLS

The vessel walls at first consist only of endothelium, and at this stage the mesenchyme cells next to the endothelium are similar to those elsewhere. In the main vessels of the embryo, this stage is soon succeeded by one in which a single layer of mesenchyme cells is flattened against the endothelium. It may be that cells of this flat mesenchyme layer are able to join the endothelium, if vessels inside the embryo grow in diameter in the same manner as extra-embryonic vessels (Hughes, 1937). The endothelial nuclei are usually elongated in the direction of the axis of the vessel, while the nuclei of the mesenchymal layer are frequently elongated along the circumference. From the fourth day onwards, other cell layers from the mesenchyme add themselves to the layer round the endothelium. When several layers of cells have joined the vessel wall they form a compact dense zone round the lumen, which is clearly distinct from the surrounding mesenchyme. The cytoplasm of the cells becomes more distinct; the cell nuclei may either remain flattened and elongated, or they may become rounded or ovoid. In a vessel whose wall consists of several cell layers, the cells lose their original flatness and the wall increases markedly in thickness. This change takes place in the main vessels of the embryo between 5 and 7 days.

The first intercellular fibres to appear in the vessel wall are the reticular or argyrophil fibrils, which are revealed by silver impregnation methods such as Wilder's (Wilder, 1935). The density of these fibres in the vessel wall is proportional to their density in the surrounding mesenchyme. Thus, in the embryonic membranes, where the development of reticular fibres is much ahead of that inside the embryo, the extra-embryonic vessels, both arteries and veins, acquire well-developed reticular fibres before these appear in the main vessels of the embryo. Thus at 4 days both the general mesenchyme of the embryo and the cell layers round the aorta after silver impregnation show only the faintest trace of reticular fibrils, which in the vitelline arteries and veins are well developed at this stage. The vessels of the allantois, as yet only of capillary dimensions, are provided with fine fibrils.

At 5 days reticular fibrils are recognizable in the general mesenchyme of the embryo, and in the cell layers round the main vessels. They gradually become thicker, and by 7 days the thickest fibres in the vessel walls are considerably stouter than the fibres of the general mesenchyme. Between the fifth and seventh day the first elastic fibres appear in certain places (Pl. 1, figs. 5, 6 and 8). The earliest embryo in which I have seen a positive reaction with Weigert's elastin stain is one of 5½ days, in which the left fourth arch was becoming noticeably smaller than the right. In the loose mesenchyme at the base of the aortic arches, between the common aortic and common pulmonary trunks, distinct purple granules are to be seen, not yet joined into continuous fibres. At 7 days elastic fibres are seen further distally, at the level where the common trunks have split into their component aortic arches. At this level, in addition to the rows of elastin granules between the loose mesenchyme round the arches, we see the cell boundaries between the

closely packed cells of the aortic arch walls outlined in thin membranes of elastin. The outer cell layers of the aortic arches are better provided with elastin than the inner, and the membranous form of elastin between the closely packed cell layers graduates into the loose granular form in the mesenchyme beyond (Pl. 1, fig. 8).

In general, elastin first appears near the heart, and is found more and more distally as development proceeds. To this rule there is one striking exception which is described below.

Silver impregnation by Wilder's method reveals both the reticular fibres which are the forerunners of collagen fibres, and also elastic fibres. The finest elastic fibres, which appear as rows of granules when stained with Weigert's stain, are not well revealed by silver impregnation; only the older fibres which have passed the granular stage are clearly seen in Wilder preparations. Consequently, after silver impregnation the larger fibres seen may be either elastic or young collagen fibres. Only the largest collagen fibres from embryos in late stages of incubation are unaffected by silver impregnation. Aniline blue stains both collagen and elastin, and the only specific fibre stains are the elastin stains, Weigert's and orcein.

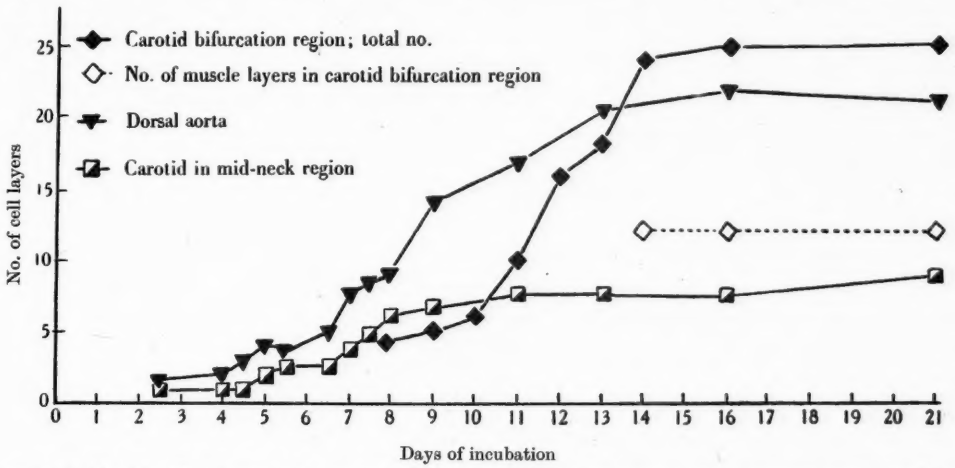
The morphological changes which have been described in the preceding section begin just at the time when the walls of the main vessels have reached the stage at which external cell layers are beginning to surround the endothelium closely. It is of interest to examine whether either the rapid changes in length of the dorsal vessels or the secondary splitting of the median aorta affect in any way the laying down of cell layers round the endothelium.

It might, for instance, be expected that rapid shortening or lengthening of a vessel would affect the rate at which this process takes place. Yet both in the carotids and in the median aorta the cell layer number increases regularly throughout the period when the aortic arches are migrating and for some days after this period (Text-fig. 2).

Thus as the carotid elongates, the total number of cells comprising the whole carotid wall must increase at the same rate as the vessel increases in length. The aorta behind the arches for a short time rapidly diminishes in length, while the number of cell layers in the wall is continuously increasing. At this time, the total number of cells in the aortic wall is probably diminishing, as the decrease in length probably outweighs the general increase in thickness. It is possible that cells may be lost from the aortic wall by re-entry into the general mesenchyme.

Whether this is so or not, a special site where cells are being destroyed is found in the aortic wall at the time of shortening. At 5 days of incubation, when secondary bifurcation of the aorta begins posterior to the pulmonary arches, a localized region of cell degeneration appears at the point of bifurcation (Pl. 1, fig. 1; Text-fig. 1*a*). At 5 days backward migration of the arches has already begun; the localized patch of degeneration disappears after 7 days, 2 days before backward migration of the arches is complete.

Before the 5-day stage, at the point of bifurcation of the aorta, there is an accumulation of cells denser than elsewhere in the aortic wall. At 5 days pycnosis appears in the cell nuclei of the dorsal part of the dense mesoderm, and very soon is so wide-

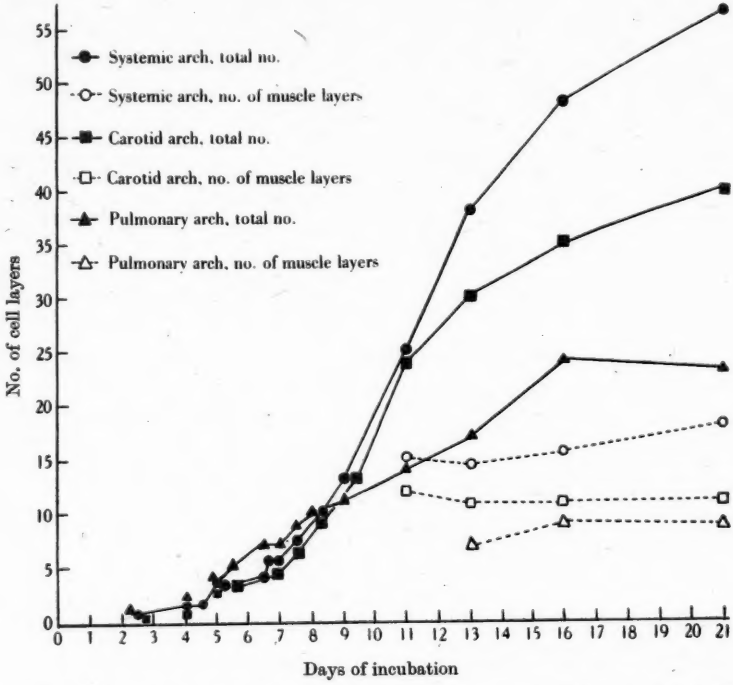

Carotid bifurcation region; total no.
No. of muscle layers in carotid bifurcation region
Dorsal aorta
Carotid in mid-neck region
No. of cell layers
0
5
10
15
20
25
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days of incubation

Text-fig. 2. Number of cell layers in the tunica media of the median dorsal aorta and the common carotid arteries in mid-neck region, and in the region of the carotid bifurcation (plotted against days of incubation).

spread that a localized area of necrosis is formed of the same order of size as the mid-ventral zone of the body wall, which has been described in connexion with the development of the avian sternum (Fell, 1939).

At 6½ days the necrotic area is not limited to the upper part of the dense mesenchyme at the bifurcation, but the density of pycnotic nuclei is less than at 5 days, and the end stages of degeneration predominate (Pl. 1, fig. 2). At 7 days, when the point of bifurcation is now in the final position much better developed here than elsewhere in the aortic wall.

This degenerate area appears when two morphological processes are taking place in the aorta, namely, shortening and secondary bifurcation. The degenerate area may be a localized region for the destruction of cells of the aortic wall rendered superfluous by the decrease in length of the aorta, or, on the other hand, it may be the consequence of the splitting of the aorta into two divisions. It is possible to determine whether the splitting of a



Text-fig. 3. Number of cell layers in the tunica media of the aortic arches at points half-way along the arches (plotted against days of incubation).

relative to the median aorta beyond, cell degeneration has very largely disappeared save for an occasional end stage. At the point where the median aorta bifurcates, and which up to the 7-day stage is a localized area of cell degeneration, elastic fibres first appear well in advance of their being formed elsewhere in this region. As soon as cell degeneration ceases at this point, elastic fibres appear, and remain isolated until after the 8-day stage. It may be that the cells which degenerate leave behind them a substrate rich in the substances from which elastic fibres are formed. At stages when pycnotic nuclei are found in this region, reticular fibrils are vessel into two divisions necessarily causes the appearance of cell degeneration at the point of bifurcation by studying the tertiary bifurcation of the right-hand division, which separates the right ductus arteriosus from the systemic duct. Here, a dense mass of mesoderm is also formed at the fork, but no general cell degeneration occurs. This fact supports the view that the degenerate area is a mechanism for the destruction of superfluous cells in the shortening aortic wall (Pl. 1, fig. 7).

Since we can trace a connexion between cell degeneration and shortening of a vessel, we might expect to see cell degeneration in the wall of a vessel

which is disappearing altogether. Three pairs of aortic arches disappear during development, namely the first, second and the transitory fifth, as well as the left fourth arch at a later stage. At the stages of development when the first two pairs of aortic arches are disappearing, vessel walls consist of endothelium only with no closely related mesenchyme layers beyond. I have been unable to find any pycnotic nuclei in the first and second visceral arches of embryos at stages shortly after the disappearance of the first and second pairs of aortic arches. In general, cell degeneration does not occur in embryonic endothelium. The endothelium of the first and second arches may dedifferentiate into mesenchyme, or, since an extensive capillary plexus succeeds the first and second aortic arches, their endothelium may undergo rearrangement as a capillary plexus.

The left fourth arch begins to decrease rapidly in diameter during the fifth day, when several layers of cells have surrounded the main vessels. The aortic arches on the right side are always somewhat larger in diameter than those on the left, for, as Kranichfeld (1914) has shown, the bulbus of the heart points towards the right side, and so the right arches provide a more direct path than those on the left side. In the 5-day embryo from which Text-fig. 1*a* was reconstructed, the diameter of the right fourth arch is almost twice that of the left. Both fourth arches have two to three layers of cells applied to the endothelium. In the 6½-day embryo reconstructed in Text-fig. 1*b*, the left fourth arch has decreased further in diameter by one-half, while the wall is still similar to that at 5 days and shows no degeneration. In a slightly older embryo, where the lumen has reached capillary dimensions and is blocked by aggregated blood corpuscles, pycnotic nuclei appear both among the cells outside the endothelium and within the lumen. It is not possible to say whether any endothelial nuclei are pycnotic (Pl. 1, fig. 4). Thus the cells in the wall do not degenerate until the last stages of shrinkage in the diameter of a vessel. During the earlier phases cells presumably leave the wall, and they rejoin the surrounding mesenchyme since the number of cell layers remains the same. The same course of events is seen in the disappearance of the ductus caroticus. In the 5½-day embryo of Text-fig. 1*a* the wall of the ductus caroticus on each side consists of four to five cell layers surrounding the endothelium, and the diameters of left and right ductus caroticus are respectively 90 and 175 μ. In the 6½-day embryo of Text-fig. 1*b*, the respective diameters are now 33 and 90 μ, indicating that many cells have left the wall, since no degeneration has yet appeared (Pl. 1, fig. 7). In later embryos, in which the ductus caroticus is no longer an open vessel on either side, pycnotic nuclei are found within it. The right ductus caroticus seems to degenerate more rapidly than the left; in the 7½-day embryo reconstructed in Text-fig. 1*c*, a tract of degenerate cells is traceable from the apex of the carotid arch to the pulmonary arch on the left side, but not on the right (Pl. 1, fig. 4).

THE MAIN TYPES OF ARTERIAL WALLS

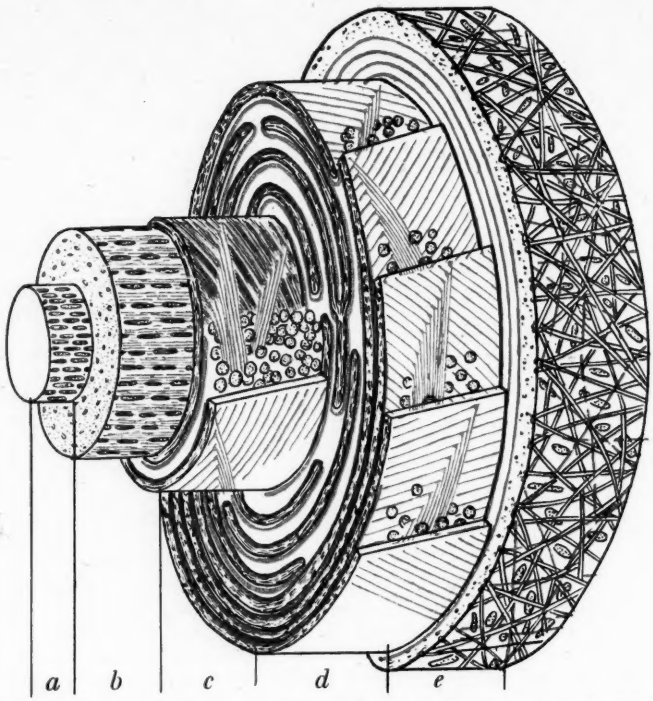
Up to the seventh day the histogenesis of the vessel walls has advanced no farther than the differentiation round the endothelium of cell layers which are destined to belong to the tunica media. These cells are recognizably different from those of the mesenchyme. The first signs of further differentiation within the vessel wall appear after the seventh day, first at the base of the aortic arches, and farther and farther distally in successive stages.

To describe the histogenesis of the vessel walls, it is first necessary to consider the final structure towards which development proceeds. This is the order of description which has been adopted in the succeeding sections of this paper. The structure first described in each section is that of the particular vessel in the chick at the hatching stage. Important changes in arterial structure take place in subsequent stages of the life period, but they are more quantitative than qualitative, and can be more profitably discussed in a future paper.

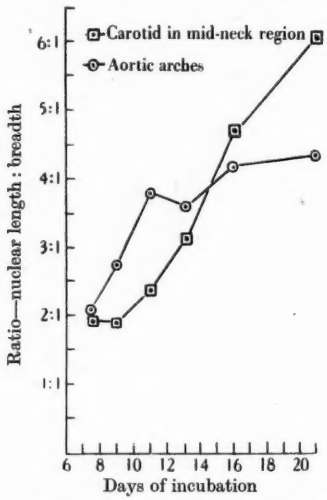
Three main types of arterial structure in the chick have been distinguished, which are known as types I, II and III. Of these, the first two are types of elastic arteries and the third is that of the peripheral muscular arteries. Type I refers to the aortic arches, which are succeeded distally by arteries of type II which is the intermediate between types I and III. The two types of elastic artery differ profoundly in structure. Type II has more in common with type III than with type I, and for this reason, among others which are discussed later, the ordinary classification of arteries as either 'elastic' or 'muscular' has not been used here (Text-figs. 4, 7 and 8).

Type I. The distinguishing characteristics of the type I structure are as follows: the greater part of the tunica media is made up of alternate layers of muscle fibres and layers of non-muscular cells in between which run layers of elastic fibres. The muscle fibres in general are orientated obliquely. Next to the endothelium is an intimal zone of several cell layers in between which longitudinal elastic fibres are found. The adventitia is poorly developed, and consists of one or two layers of cells in between which run bundles of collagen fibres (Text-fig. 4).

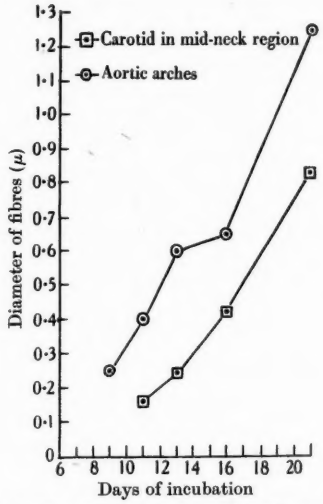
The muscular layers do not form complete concentric cylinders, for in both transverse and longitudinal sections it can be seen that muscle layers stop short, or bifurcate, or run into adjacent muscle layers (Pl. 2, figs. 10–12). In transverse sections it appears that each continuous muscle layer does not



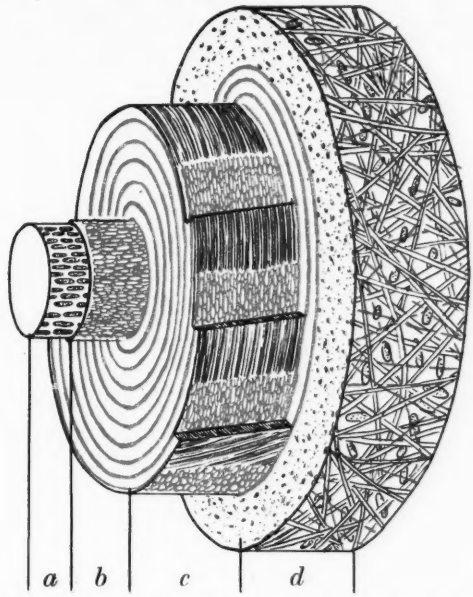
Text-fig. 4. Diagram of the fully developed structure of the aortic arches (type I) to show the following zones: (*a*) the endothelium, (*b*) the intimal zone, (*c*) the inner and middle muscular zones of alternate muscular and elastic layers, (*d*) the outer layers of the tunica media, where the elastic layers are much thinner and the orientation of the muscle fibres approaches the longitudinal direction, (*e*) the thin adventitia. Elastic fibres indicated in red.



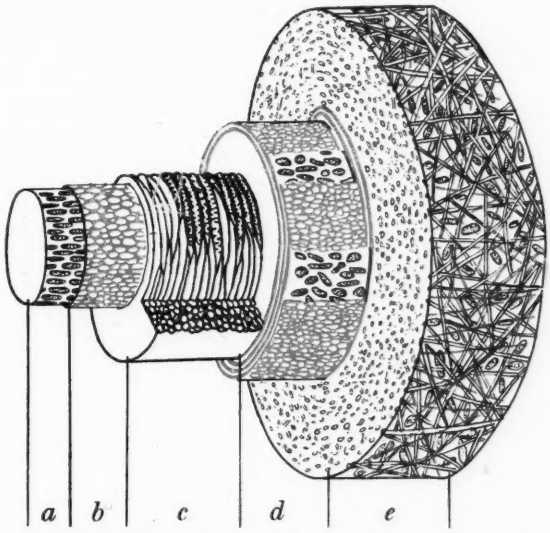
Text-fig. 5. Ratio of nuclear length to breadth plotted against days of incubation for the aortic arches and for the common carotid arteries.



Text-fig. 6. The diameter of the elastic fibres of the aortic arches (thickest fibres), and of the external elastic region of the common carotids (plotted against days of incubation).



Text-fig. 7. Diagram of the fully developed type II arterial structure to show the following zones: (*a*) the endothelium, (*b*) the elastica interna, (*c*) the tunica media, consisting of alternate elastic networks and muscle fibre layers, circumferential except the outermost layers, and (*d*) the adventitia. Elastic fibres indicated in red.



Text-fig. 8. Diagram of the fully developed structure of a peripheral artery (type III) to show the following zones: (*a*) the endothelium, (*b*) the elastica interna, (*c*) the tunica media, consisting of circular muscle fibres, not arranged in regular layers and surrounded by reticular sheaths, and with occasional elastic fibres between them, (*d*) the elastica externa region, consisting of several elastic networks, and (*e*) the thick adventitia. Elastic fibres indicated in red.

traverse more than one-quarter of the circumference. Consequently, the obliquely running muscle layers do not describe complete spirals in the vessel wall. This must be borne in mind in using the term 'layer' for these incomplete cylinders of continuous muscle fibres. All the muscle fibres within each layer run in the same direction, and in general the orientation in adjacent layers is different. In a median longitudinal section, the obliquity of orientation of a muscle layer is revealed by the longitudinal shift of the outlines of the nuclei of the muscle fibres when by using the fine adjustment of the microscope we traverse that part of the vessel wall which is included between the two planes of section. Under these circumstances, the shift of the nuclei in adjacent muscle layers can usually be seen to be in opposite directions. The most useful plane of section for the study of the orientation of successive muscle layers is at a small angle to the axis of the vessel, so that the cut surface of the vessel is a much elongated ellipse. Along the major axis of this ellipse we can trace through successive layers of the vessel wall, and the orientation of cells and fibres will appear substantially as if seen in a plane parallel to the vessel axis (Pl. 2, fig. 12).

If suitably oblique sections are chosen which traverse the whole thickness of the vessel wall, it can be seen that the outer muscle layers are orientated either longitudinally, or at a small angle to the longitudinal plane, while the inner muscle layers are orientated transversely. The longitudinally orientated intimal layer contains no muscle fibres. Starting from the nearly longitudinal outer muscle layers, and passing inwards to the inner transverse layers, the change in angle of orientation is a gradual one. Some of the layers, however, follow a left-handed spiral, and others a right-handed spiral, and in the fully developed system there is a strong tendency for left- and right-handed spirals to alternate regularly with each other. Sometimes two adjacent layers will point in the same direction, but in general layers pointing in each direction are present in equal numbers. Especially in the pulmonary arch, where the total number of muscle layers is small (under ten), the regular alternate arrangement stands out very clearly. In the systemic and carotid arches alternation is more regular in the outer layers.

Between the muscle layers are found the non-muscular or elastic layers, which consist of layers of cells with rounded nuclei, up to three or four in number, between which run complex and highly developed elastic fibre systems. In the systemic and carotid arches at the end of development the elastic layers are very much more conspicuous than the muscular layers (Pl. 2, fig. 11). Elastic layers three or four cells thick are only found in the inner half of the wall in the systemic and carotid arches. Between the outer muscular layers the elastic layers are only one cell thick. The total number of layers of non-muscular cells is considerably greater than the total number of muscular layers.

Oblique sections through the wall are also the most useful for the study of the relationships between the elastic and muscular layers (Pl. 2, fig. 14). The elastic layers consist of several main sheets of parallel fibres. Applied to the surface of all muscle layers are close coverings of elastic fibres running in the same direction as the muscle fibres. The elastic fibres applied to two adjacent muscle layer surfaces are linked together by bundles of stout elastic fibres which run across each non-muscular layer. Each non-muscular layer, therefore, contains elastic fibres in three orientations, namely two sets of fibres with the same orientation as the adjacent muscle layers, and a third set running between them, usually transversely orientated (Text-fig. 4).

When the state of contraction of two adjacent sets of oblique muscle fibres of opposite orientation is altered, the transverse shift relative to each other is countered by the action of the elastic fibre system between them. The oblique muscle fibre layers distribute stresses evenly between circular and longitudinal components, and the elastic layers transmit the stresses originating in the inner layers of the vessel wall successively to the outer layers.

The elastic fibres anchored on to the surface of the muscle layers do not appear to penetrate between the muscle fibres themselves. After silver impregnation by Wilder's method a network of reticular fibres is seen on the muscle layer surface in addition to the fine elastic fibres, and this network also does not penetrate between the muscle fibres.

The intimal zone at the end of development consists of up to ten layers of non-muscular cells, between which elastic fibres run longitudinally. The fibres nearest the endothelium are finer than those in the outer part of the intimal layer. Unlike the cells in the elastic layers between the muscular layers, the cells of the intimal layer have elongated nuclei. They point in the same direction as the muscle fibres, and are very similar in appearance to the endothelial nuclei themselves (Pl. 2, fig. 13).

The adventitia is represented by one or two layers of cells outside the muscular layers, in between which run thick collagen fibres. It is very inconspicuous in relation to the other layers of the vessel wall.

Type II. The distinguishing characteristics of the type II structure are as follows: the tunica media is composed of muscle fibre layers alternating with elastic laminae; both muscle fibres and elastic fibres run circumferentially. Each of the elastic laminae consists of circumferential fibres which at fixation are thrown into longitudinal folds; the latter present a wavy appearance in transverse section.

The endothelial cells are longitudinally orien-

tated, and immediately outside the endothelium is found a layer of longitudinal elastic fibres, which may be termed the tunica elastica interna. In places the tunica elastica interna may consist of two layers, but the thick longitudinal intimal layer of the type I structure is not developed.

The tunica adventitia is more conspicuous than in arteries of type I, and contains at least four layers of cells with elongated nuclei, in between which run thick bundles of collagen fibres. Fine elastic fibres are also found in the tunica adventitia.

The majority of the muscle fibres run transversely, but in the two or three outer muscular layers the orientation is oblique or even longitudinal. The elastic fibres follow the orientation of the adjacent muscle layers (Text-fig. 7).

Analysis of the structure of the elastic laminae is rendered difficult by the longitudinal folding produced at fixation. Thus in longitudinal sections the portions of these folds which run radially are at right angles to the plane of section, and appear as very strongly marked longitudinal lines. The direction of fibre orientation must be followed in those parts of the laminae which are seen in surface view in oblique longitudinal sections, namely the crests and troughs of the undulations. Despite these difficulties, certain features in the structure of the laminae are clearly distinguishable. All laminae, with the exception of the tunica elastica interna, consist of close fibres, generally running transversely. Some of the fibres do not follow the folds in the lamina, but run alongside the muscle fibres, sometimes penetrating between them.

The tunica elastica interna, although longitudinally orientated, does not consist of separate fibres, but is a network whose meshes are very much elongated in the longitudinal direction.

In some arteries of type II structure we see the beginnings of a distinction between the inner and outer halves of the tunica media, in that the elastic laminae in the outer half are more strongly developed than in the inner half, and that in the outer half there are some non-muscular cells alongside the muscle cells. These non-muscular cells in the outer half of the tunica media have nuclei which are either rounded or irregular in shape. The separation of the tunica media into an inner and an outer half marks the transition from the type II structure to the type III structure.

Type III. In the arteries of type III, the separation of the tunica media into two halves has proceeded so far that no muscle fibres remain in the outer half, and in the inner half elastic fibres may very largely disappear, leaving an isolated tunica elastica interna (Text-fig. 8).

The outer half of the tunica media becomes a tunica elastica externa consisting of several layers of elastic laminae between which non-muscular cells are found (Pl. 4, figs. 26, 27). These elastic laminae undergo longitudinal folding at fixation, where contraction is not artificially prevented. In arteries fixed in a stretched condition it can be seen that the laminae of the elastica externa consist of networks of elastic fibres whose meshes enclose rounded spaces. The cells in between the elastic laminae have elongated nuclei which are generally orientated in the longitudinal direction.

In the muscular part of the tunica media the elastic fibre component undergoes varying degrees of reduction. Fine elastic laminae may alternate regularly with the muscle fibre layers, or there may be one or two fine laminae to a larger number of muscular layers. The laminae within the muscular zone are networks, similar to the tunica elastica interna and externa. In addition, single elastic fibres often run with the muscle fibres.

The muscle fibres are much more closely bound to each other in arteries of type III than in the other types of artery. In longitudinal sections of type III arteries from post-embryonic stages the outlines of the muscle fibres, cut in transverse section, are marked by sheaths of collagen, staining deeply with aniline blue. Seen in surface view, these sheaths appear as a coarse reticulum.

In arteries of type III at the hatching stage, silver impregnation by Wilder's method reveals fine reticular sheaths round the muscle fibres, best developed in the outer part of the muscular zone. These fine sheaths do not yet stain with aniline blue (Pl. 4, fig. 30).

The orientation of the muscle fibres is similar to that of the arteries of type II, namely mostly transverse, with a tendency towards obliquity in the outer layers.

The tunica adventitia reaches its highest state of development in arteries of type III. It is thicker and contains more cell layers than the rest of the arterial wall. The cells of the tunica adventitia have elongated nuclei with somewhat irregular outlines. Fine cytoplasmic processes ramify among the bundles of collagen fibres.

The endothelium in arteries of type III differs in appearance from that in arteries of other types. The endothelium shrinks away from the elastica interna and the endothelial cells stand out distinctly from one another, and there is a pronounced tendency for the vacuolization of the endothelial cytoplasm. It may be that these peculiarities are artefacts bound up with the longitudinal folding of the elastica interna.

HISTOGENESIS OF THE MAIN VESSELS

As a numerical index of the growth in thickness of the walls of the main arteries at these stages, I have used the number of layers of cells which are packed closely round the endothelium and are thereby different in appearance from the surrounding mesen-

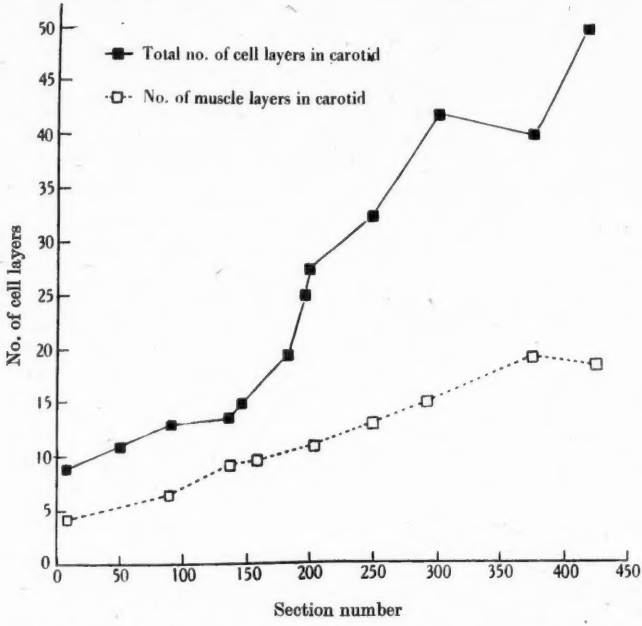
chyme from which in the first place they are derived. The same index is also used in stages where the cell layers of the vessel wall are themselves becoming differentiated, and in which the final structure of the vessel wall is appearing (Text-figs. 2, 3, 9, 10 and 11).

The dense cell layers round the endothelium represent the future tunica media. The cells of the mesenchyme immediately beyond may in future stages either add themselves to the tunica media, or may form the tunica adventitia. The cell layer counts which are plotted in these text-figures in all cases refer only to the tunica media.

in the pulmonary arch than in other arches at 7½ days, but also the elastic fibres are further advanced in development (Pl. 1, fig. 9).

After the ninth day the position is suddenly reversed. The number of cell layers in the pulmonary arch has not increased since the 7½-day stage, but in the systemic arch the number of cell layers at the base has increased by 1½ times. From the 9-day stage onwards the development of the systemic arch is progressively ahead of the pulmonary arch, both in number of cell layers and in complexity of structure.

At 7½ days, the arrangement of the cell layers at



Text-fig. 9. Number of cell layers in the tunica media of the carotid arches and common carotid artery of a 1-day hatched chick plotted against distance from the origin of the carotid trunk, as measured by section number.

In later stages, where the developing tunica adventitia is recognizable, it has not been included in the cell layer count for the sake of uniformity with the counts made at earlier stages. In the arteries nearest the heart, the aortic arches (Text-fig. 3), where the tunica adventitia comprises a very small number of cell layers in comparison with the whole tunica media, the counts given in Text-fig. 3 are therefore very nearly identical with those for the whole vessel wall.

(1) *The development of the aortic arches.* In the earlier section dealing with the number of cell layers in the arches at different stages, the early predominance of the pulmonary arch was pointed out and discussed. Not only are there more cell layers

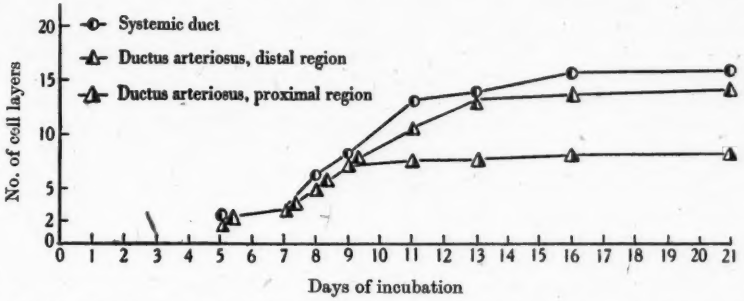
the base of the arterial arches already foreshadows the future type I structure. The cell layers are separated by clear spaces, in some of which already run elastic fibres (Pl. 1, fig. 6). These cell layers in transverse section appear as arcs of continuous cells which, with ordinary histological stains, give the appearance of a continuous layer of homogeneous cytoplasm in which nuclei are embedded at intervals. Closer study of these layers of myoblasts by cytological methods has not been made for the purposes of the present investigation. These arcs of continuous myoblasts usually extend for less than one-quarter of the circumference, when they either end blindly or join with an adjacent myoblast layer. Round the endothelium are one or two layers

of cells not differentiated as myoblast layers, which foreshadow the future intimal layer.

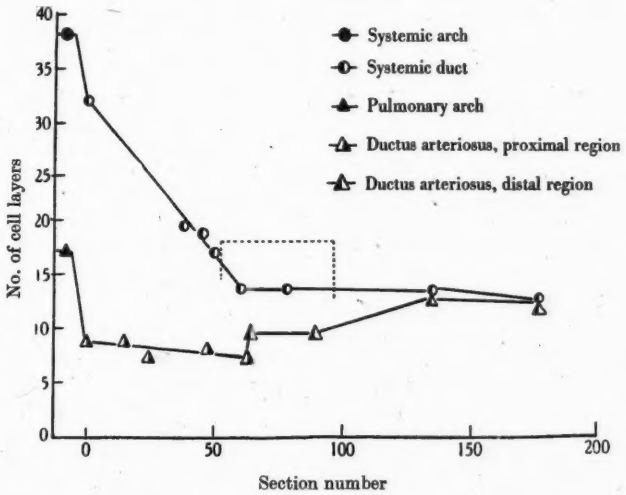
The outer myoblast layers are not so widely separated from each other as are the inner layers. This distinction between outer and inner layers is probably to be explained by a greater degree of contraction at fixation in the inner layers. This is one of several artefacts which are produced by fixation.

To serve as an index of the differentiation of the myoblasts into smooth muscle cells, the ratio of nuclear length to breadth has been measured at different stages. Ten nuclei were chosen at random, and the average ratio for each stage has been plotted against time of development (Text-fig. 5). The curve rises very steeply at first, and later, towards the third week of development, the ratio increases much less rapidly. The curves for the number of cell layers are similar, and the implications of these results will be discussed later.

The number of myoblast layers at the base of the systemic arch at 7½ days is about half the full number of muscle layers present there at the end of development. The full number is first acquired between the ninth and eleventh days of incubation. In the pulmonary arch the full number is already present at 7½ days. The cells subsequently added to the walls of the arch are non-muscular and consist



Text-fig. 10. Number of cell layers in the tunica media of the systemic duct and ductus arteriosus in both proximal and distal regions plotted against days of incubation.



Text-fig. 11. Number of cell layers in the tunica media of the systemic duct and ductus arteriosus of a 13-day embryo plotted against section number. The left side begins with the respective aortic arches and the right side ends with the fusion of systemic duct and ductus arteriosus. The part of the systemic duct curve enclosed by the dotted line refers to the region where the arrangement of the elastic fibres is disturbed by the entry of neuroblast cells.

of the intimal zone adjacent to the endothelium, and the cells of the elastic layers between the muscular sheets. The intimal zone is already suggested at 7½ days, but the cells of the elastic layers are first seen at 11 days, when they appear in the inner part of the systemic arch. They are probably derived from myoblast cells which leave their respective layers to take up positions between adjacent myoblast sheets. At 11 days mitotic figures among the myoblasts are very common indeed.

At 13 days, in the systemic arch, cells are present in all elastic layers between the muscular layers. The inner elastic layers are becoming more than one cell thick. In the pulmonary arch such cells are only just appearing. In all subsequent stages the total number of cell layers in the elastic layers is less than the number of muscle sheets, while in the systemic arch the number is very much greater.

The growth of the intimal zone proceeds on lines similar to those of the other non-muscular layers. In the carotid and systemic arches the zone develops earlier and proceeds farther than in the pulmonary arch, and new cell layers are still being added to it after the stage when the arch has acquired its full number of muscular layers. At 7½ days the two cell layers next to the endothelium suggest the future intimal zone in that they consist of discrete cells; the nuclei at this stage tend towards a radial orientation. Elastic fibres first appear among the cells next the endothelium at 9 days. These fibres are extremely fine and are longitudinally orientated. In the pulmonary arch at 9 days the myoblast layers reach inwards as far as the endothelium, indicating that the cells investing the endothelium at 7½ days have differentiated into myoblasts. A true intimal zone of one to two cell layers first appears in the pulmonary arch at 11 days; at this stage in the other arches the intimal layer consists of two to three cell layers. In the carotid and systemic arches contraction during fixation has resulted in longitudinal foldings of the endothelium, which give the lumen a star-shaped appearance in transverse section. At the points of this star, the longitudinal fold of endothelium projects into the intimal layer as far as the inner muscle layer. The intimal zone is heaped up between these folds. This distortion is due to the extreme contraction of the inner muscle layers at fixation, producing at the same time an exaggerated separation of the muscular layers, as already mentioned. At 11 days the endothelium of the pulmonary arch is not folded in this manner, but a slight folding is found from 16 days onwards.

This endothelial folding prevents an accurate count being made of the number of cell layers in the intimal zone, but it is clear that they continually increase in number during the incubation period. At 21 days the number in the pulmonary arch is about half that in the systemic arch. The orientation of the elastic fibres is uniformly longitudinal, as is the direction of elongation of the intimal cell nuclei. The elastic fibres near the endothelium are always finer than those nearer the muscle layers (Pl. 2, fig. 13).

The collagen fibres in the rudimentary adventitia do not develop until after the sixteenth day of incubation.

The next topic to be discussed in this section is that of the development of the orientation of the muscle fibres and elastic fibres, as seen in oblique sections through the arches at different stages. At 7½ days a constant orientation can be traced in the outer layers only, where the nuclei tend to be orientated longitudinally. In those regions of the arches which are cut longitudinally in sections transverse to the embryonic axis (i.e. where the arches run upwards to the dorsal vessels), elastic fibres have not yet appeared at 7½ days. At 9 days we can recognize certain tendencies in the orientation of myoblast nuclei in the deeper layers. Myoblast nuclei in the middle zone of the wall tend to be orientated transversely. This orientation changes gradually to the longitudinal one in passing to the outer layers. The orientation of the inner muscle layers tends to be longitudinal also and grades into that of the intimal layer. Thus at 9 days the main orientations characteristic of the fully developed type I structure are already present. Two differences from the final structure can be recognized: first, that there is as yet no sharp distinction between the inner transverse muscle layers and the longitudinally orientated non-muscular intima which is characteristic of the fully developed type I structure, and secondly, there are as yet no signs of the future alternation of right- and left-handed layers.

Some alternating layers appear at the 11-day stage (Text-fig. 6), and at 16 days the wall of the pulmonary arch forms a fully alternating system (Pl. 2, fig. 12). At 16 days there are still a few remaining longitudinal muscle fibres near the intimal layer, but they are immediately succeeded in the next muscle layer by transverse fibres. Most of the remaining longitudinal fibres are of a type which is much narrower and more densely staining than the majority of muscle fibres and are very common in all layers at 16 days. The nuclei are long and narrow, being greater in length and about one-quarter the breadth of the nucleus in other muscle fibres. Both nucleus and cytoplasm stain extremely densely. They stand out very clearly from the other muscle fibres.

The first muscle fibres to differentiate from myoblasts are of this type, and occasionally such fibres are seen at 9 days. At 21 days they have entirely disappeared. It seems probable that they represent precocious developing muscle fibres which degenerate towards the end of development. The appearance of many of them at 16 days suggests that they

are in process of degeneration, as does the high proportion of fibres of this type among the longitudinal muscle fibres near the intima, where such fibres are not found at 21 days.

We now pass to the differentiation of the elastic fibres in the non-muscular layers, and their relationship to the muscle fibres. At 9 days, networks of elastic fibres are present between all myoblast layers and between the cell layers bordering the endothelium. The elastic fibres nearest the lumen are the thinnest, and presumably the youngest. All fibre layers at this stage consist of a network in one plane only; that is, when observing a fibre network in a plane parallel to the stage of the microscope, it is not possible to see other fibres of the same network by focusing upwards or downwards. The only fibre layers at this stage with a definite orientation are the innermost ones, which run longitudinally as they do in all subsequent stages. All other layers consist of networks of wavy fibres which anastomose with each other.

At 11 days the elastic networks have differentiated so far that different types of fibres can be recognized in each. Most form part of a general meshwork, in which no predominant orientation can be traced. In addition, however, we see two sets of fibres which do not form networks, and of which all the fibres in each set are arranged parallel to one another. One set consists of very fine fibres closely applied to the surfaces of muscle layers, with the same orientation as the muscle layer which they cover. They do not appear to penetrate between the muscle fibres themselves. The other set consists of bundles of a few coarse fibres, which run for considerable distances linking together the elastic fibres covering two adjacent muscle layer surfaces. These two sets of orientated fibres represent the final arrangement of the elastic layers found at 21 days.

The subsequent changes in the elastic layers are twofold. In the first place, the individual elastic fibres increase steadily in thickness. Their thickness at different stages was measured by means of an eyepiece micrometer under a magnification of 1505 diameters at which 1 division of the micrometer represents 0·83 μ. The diameter of early elastic fibres up to 9 days is about 0·25 μ, while at 21 days the diameter of the thickest fibres is 1·5 μ. The increase in thickness of the elastic fibres from 9 to 21 days appears to be linear (Text-fig. 6).

The second change in the elastic fibre networks after 9 days is the increase in the importance of the two sets of orientated fibres in comparison with the general fibre network. From 16 days onwards the surface of the muscle layers has very heavy coverings of parallel elastic fibres which are tied together with stout fibre bundles (Pl. 2, fig. 14). The general fibre reticulum becomes inconspicuous. The fine reticular network on the surface of the muscle layers revealed by silver impregnation does not develop until after 13 days of incubation.

(2) *The carotid arch and common carotid artery.* The carotid arch, in common with the other aortic arches, is of type I structure. As we pass forwards distally from the arch, we find that the arch structure persists to a short distance in front of the origin of the subclavian artery, where both the continuation of the main stem and the subclavian artery soon change to the type II structure which in the former persists until the vertebral artery has been given off. This is of type III, and forwards from this point the common carotid itself rapidly becomes an artery of type III.

The structure of the common carotid for the rest of its course remains constant until just before the bifurcation into external and internal carotids when it suddenly changes back to type I (Pl. 3, figs. 18, 19). The existence of a vessel of this type in so peripheral a position complicates very greatly the interpretation of the functional interrelationships of the three types of artery. To the development of the type I structure at the carotid bifurcation we shall return later. At present, however, we are concerned solely with the proximal region of the carotid arch and artery.

The total number of cell layers in arteries of type I in general is considerably greater than in arteries of types II and III. At the point of transition between the type I structure and the type II structure, the number of cell layers does not suddenly alter, for the number of cell layers in the arch gradually decreases right from the beginning as we pass distally. In Text-fig. 9 the number of cell layers in the carotid arch has been plotted for different distances from the point where the right and left carotid arches are given off from the carotid root. (Since in sections transverse to the body axis the carotid arches are also cut approximately transversely in stages after the first week of development, the distances along the carotid in Text-fig. 9 are obtained from the numbers of sections in the section series.) Text-fig. 9 shows that the number of muscular layers in the carotid arch and artery declines steadily as far distally as the type III region, and that the total number of cell layers falls more rapidly, but less regularly. For the type I section, the decrease is principally in the number of non-muscular layers, both in the intimal layer and in the intermuscular layers. The intimal thickening is the first distinctive type I feature to be lost as we pass distally. At the point where no more intimal layers are left most of the muscular layers are transverse in orientation, and as the elastic layers further distally gradually become more compact, the type II structure is reached.

After the origin of the vertebral artery, all muscle cells disappear from the outer layers of the tunica media, and the elastic laminae between the mus-

cular layers of the inner part of the tunica media become much finer. Farther distally, some of these fine laminae disappear. The number of muscle layers decreases by about half as we pass from the type II section to that of type III, while the number of non-muscular layers remains the same, although now confined to the elastica externa region and adventitia.

The appearance of the future type I structure at 7½ days has already been described in a previous section. In the carotid arch distinct myoblast layers with spaces between them can be traced for two-thirds of the way to the subclavian arteries. At 9 days distinct myoblast layers can be traced as far forwards as this point, and at 11 days to a point some way beyond, the limits of the type I structure being then the same as at the end of development.

At 7½ days the carotid in front of the region recognizable as of future type I structure consists of about seven layers of undifferentiated cells. The appearance of fine elastic fibres among these cells is the first sign of differentiation of the vessel wall in these regions.

As in all arteries elastic fibres first appear in the outer cell layers and soon afterwards spread to the inner layers. At 8 days elastic fibres can be traced forwards to a point in front of the origin of the subclavian arteries, that is, to a region of future type II structure. At this stage elastic fibres are found only in the outer part of the wall, but at 9 days fine elastic fibres occur between the inner cell layers as well. At 11 days in this region, the regular alternation of well-developed elastic laminae and cell layers throughout most of the tunica media shows clearly that a structure of type II is developing. In front of the origin of the vertebral arteries, where the final structure is of type III, very fine elastic fibres are first seen at 9 days. At 11 days, in the outer part of the young tunica media, the elastic fibres are evidently further advanced, and at 13 days there is a sharp distinction between the elastic fibres in the two regions of the tunica media. Furthermore, the cells in the two regions are plainly distinguishable from each other; in the inner zone the muscle cells are as well differentiated as in more proximal regions of the artery.

The cell layers from which the adventitia will differentiate can be traced from 11 days onwards, when beyond the tunica media are cells with elongated nuclei orientated round the vessel, but separated widely from each other. These can be seen in all regions of the vessel, but are more obvious as we pass peripherally. At 13 days these elongated cells have drawn together round the tunica media, and collagen fibres can now be seen running between them. After 13 days the number of cell layers and the size of the collagen bundles increase steadily in the adventitia. Fine elastic fibres are seen in the adventitia from 13 days onwards.

(3) *The systemic arch and the systemic duct.* The morphology of the systemic duct has already been discussed in a previous section, where it was shown that it originates distally by secondary splitting of the median aorta, and proximally by continuation of the systemic arch. There is no histological distinction between the regions of the systemic duct of separate origin at any stage of development.

At 21 days the type I structure of the systemic duct is continued as far as its posterior third, in which the structure changes to type II. The point of transition, as in the carotid arch, is marked by the final disappearance of the longitudinal intimal layer. Distal to this point, non-muscular cells between the muscle layers quickly disappear, and in the short remaining section of the systemic duct the structure is uniformly of type II. The inner three or four elastic laminae are not quite as strongly developed as the remainder, but the cell layers between the outer elastic laminae are fully muscular.

As in the other aortic arches at 7½ days, we see the first trace of the future type I structure in the systemic arch by the presence of clear spaces between the myoblast layers. This layering is lost distally very soon, and does not persist as far as that region of the systemic arch which is cut longitudinally in serial sections transverse to the embryonic axis. At 9 days the layered condition persists as far as the longitudinally cut region of the arch, and at 11 days is seen as far back as the anterior part of the horizontal systemic duct. At this stage the layered condition is well developed in the proximal part of the systemic arch, and is gradually lost as the arch turns backwards into the systemic duct. At 13 days the point of transition between type I structure and type II structure lies at the end of the first third of the systemic duct; at 16 days this point is about half-way along the systemic duct, and at 21 days it is in the last third. Thus the type I structure appears to spread progressively along the systemic duct.

Beyond the limits of the type I structure at each stage, we see a progressive differentiation of type II structure. At 9 days the whole of the systemic duct consists of layers of undifferentiated cells with rounded or oval nuclei. Fine elastic fibres have appeared between the outer cell layers. At 11 days many cell nuclei are elongated, and there are elastic fibres between all cell layers. These young elastic laminae are all equally developed, except for the inner three or four which are finer than the others. The difference between the inner laminae and the rest is seen in all subsequent stages, but is less marked at the end of development.

At all stages from 11 days onwards, sections stained for elastin show a marked interruption of the course of the elastic fibres in particular places in the region of the systemic duct immediately behind the transition point from type I to type II

structure. Close examination shows that the arrangement of the cells in these places differs markedly from elsewhere. Most of the cells have rounded nuclei, and a few are elongated in a radial direction. At 11 days several clusters of such rounded cells are seen in the upper part of the systemic duct wall, chiefly in the outer half. At 13 days these cells have spread all round the circumference of the systemic duct in a narrow zone about half-way along the duct, immediately behind the transition from type I to type II. In later stages more can be made out concerning the cells themselves. At 21 days they consist of two types: one, large cells with rounded nuclei surrounded by a clear border of cytoplasm, and the other type, smaller cells with more densely staining nuclei, elongated in a radial direction. Only very fine elastic fibres are seen within these cell clusters, running between the cells (Pl. 3, fig. 22). Both these types of cells belong to the glomal system.

(4) *The pulmonary arch and the ductus arteriosus.* In both carotid and systemic arches the change in structure distally along the arches is gradual. The pulmonary arch forms a striking contrast to the other two. As soon as the pulmonary artery has been given off, the number of cell layers abruptly decreases by more than half, and the structure changes suddenly to type II (Text-fig. 11).

The region of the ductus arteriosus immediately behind the origin of the pulmonary artery has a wall composed of fewer cell layers than the distal region near the junction with the median aorta; this proximal part of the ductus arteriosus is the first to contract at hatching. It is hoped in a subsequent paper to describe the changes which take place in the ductus arteriosus at the time of hatching; here we are concerned only with the differentiation of this specialized mechanism prior to the hatching stage, and with the problem in arterial differentiation which is presented by the ductus arteriosus.

At 7½ days, when the future type I structure can first be recognized from the spaces between the myoblast layers, the limits of the type I structure in the pulmonary arch are the same as in all subsequent stages, namely, as far distal as the origin of the pulmonary artery. Beyond this point at 7½ days the elastic fibres disappear. At 9 days the change in structure of the pulmonary arch at the point of origin of the pulmonary artery is very clearly marked (Pl. 4, fig. 23). The cell layers immediately become more closely pressed to each other and become fewer in number, resulting in a marked decrease in thickness of the wall, and the elastic fibres between the cell layers become much finer. The elastic fibres between the inner cell layers disappear at once, and those between the remaining cell layers are lost gradually, the fibres between the outer layers persisting farthest. The distal region of the ductus arteriosus at 9 days still consists of undifferentiated rounded cells.

At 11 days the whole ductus arteriosus can be recognized as a young type II artery with the same number of cell layers throughout; yet the proximal region is thinner walled due to the very marked way in which the cell layers are flattened against each other. Six to seven cell layers are present at this stage, and in subsequent stages only two cell layers are added to the proximal region, while at the distal end the number of cell layers is doubled. In Text-fig. 11 the number of cell layers in the tunica media along both ductus arteriosus and systemic duct is plotted along the length of both vessels for the 13-day stage. The sudden drop in cell layer number in the proximal part of the ductus arteriosus and the increase distally is in marked contrast to the gradual decrease along the systemic duct (Pl. 4, figs. 24, 25).

At 21 days the number of cell layers along the ductus arteriosus is substantially the same as at 13 days. The final structure is of type II, in which all cell layers are exclusively muscular, and in which the inner elastic laminae are slightly less well developed than the outer laminae. As in the systemic duct, this inequality between inner and outer laminae is more marked at 13 days than in subsequent stages. The adventitia consists of four close cell layers with bundles of collagen fibres. These cell layers beyond the tunica media can be recognized at 11 days, and later surround the tunica media more closely. Between them the collagen fibres are gradually formed.

(5) *The pulmonary artery.* The pulmonary artery belongs to type III in that the tunica media is divided into an inner part with few elastic fibres, and an outer part with well-developed elastic fibres, but differs from it in that the outer layer of the tunica media is composed of longitudinal muscle fibres together with longitudinal elastic fibres (Pl. 4, fig. 29).

In the inner part of the tunica media, the muscle fibres run transversely and are accompanied by fine elastic fibres. The elastica interna consists of a network. After hatching, when the pulmonary artery increases considerably in diameter the network of the elastica interna is seen very clearly as then only slight longitudinal folding takes place during fixation.

The pulmonary artery differentiates much later than any of the vessels which have so far been described. At 11 days its wall consists of four layers of cells in which nuclear elongation is not yet marked, and no definite orientation exists except that the nuclei of the outer layer of cells are longitudinally arranged. No elastic fibres have yet developed between these cell layers, but outside them there are five to six layers of cells, very widely separated from each other, between which abundant

reticular fibres are revealed by Wilder's silver impregnation method. Most of these cells are destined for the future adventitia. At 13 days the cell layers of the pulmonary artery are still in the same condition, except that fine elastic fibres have appeared between them, and are now seen also among the loose cell layers beyond. At 16 days the two divisions of the tunica media are distinct. The inner zone consists of three layers of transverse muscle fibres, not all the nuclei of which, however, are yet markedly elongated. Fine elastic fibres run with the muscle fibres. The outer zone consists of three layers of longitudinal muscle fibres, with longitudinal elastic fibres between them. The adventitia consists of four or five cells beyond the tunica media, between which collagen fibres are now recognizable.

At 21 days the inner transverse division of the tunica media has increased to five or six cell layers, and many mitotic figures are seen among them. There are also many muscle fibres of the thinner type with densely staining nucleus and cytoplasm, like those in the aortic arches at earlier stages. In the outer longitudinal zone, which now consists of three to four cell layers, collagen fibres staining with aniline blue occur side by side with the elastic fibres. The adventitia is more prominent than in any other type of artery so far described, and is thicker than the whole of the tunica media. It consists of up to ten cell layers.

(6) *The coronary arteries.* The coronary arteries resemble the pulmonary arteries in structure in that outside the transverse muscular part of the tunica media longitudinal muscle fibres are found. The longitudinal zone in the coronary artery, however, is not so well developed as in the pulmonary artery and consists only of one cell layer. The muscle fibres in the inner zone of the tunica media, which is composed of four to five layers of cells, are not uniformly transverse in orientation, but are frequently oblique, although no regular alternate arrangement is found. The adventitia consists of three to four cell layers. The coronary arteries like the pulmonary artery are remarkable for the late stage at which the wall differentiates. Fine elastic fibres do not appear in either until 13 days of incubation, when the wall of the coronary artery still consists of four layers of undifferentiated cells, without definite orientation. At 16 days the main features in the final arrangement can be made out.

(7) *The anterior region of the common carotid.* The wall of the common carotid artery in the fowl increases markedly in thickness just before the external carotid artery is given off. Bremer (1924) first noted this feature, which he describes in a 14-day chick embryo.

Tracing forwards the anterior region of the common carotid in section series of this stage, the wall rapidly becomes thicker at the transverse level of the second cervical ganglion and changes in structure from type III to type I. In this transition the first change is that the muscular part of the tunica media inside the elastica externa region becomes gradually thicker, and elastic fibres are found between each cell layer. Gradually, the alternate elastic and muscular layered arrangement of type I is reached, and an intimal layer makes its appearance. The elastica externa region at first remains intact (Pl. 3, fig. 20), but when muscle layers appear between the elastic laminae the change in structure to type I is complete. The adventitia becomes less conspicuous. After the bifurcation into internal and external arteries, each artery is thick-walled at its origin, but very rapidly becomes thin-walled. The internal carotid is thin-walled before the stapedial artery is given off; the external carotid is thin-walled before it divides into maxillary and facial arteries (Hughes, 1934, pls. 15 and 16). The smaller arteries which are given off from the external carotid proximal to this point, viz. the occipital artery, and the mandibular ramus of the external carotid, are thick-walled at their origin but very rapidly become thin-walled. The thin-walled peripheral arteries in the head are of type III in structure as the tunica media is divided into an inner muscular zone and an outer elastica externa region, but the thickness of the whole is very much less than in any arteries nearer the heart.

The development of the type I structure in the region of the carotid bifurcation shows certain interesting features. Up to the tenth day the common carotid is uniform in thickness from the mid-neck region to the zone of bifurcation, and at 10 days consists of about seven layers of cells round the endothelium denser than the surrounding mesenchyme (Pl. 3, fig. 16). Between the tenth and the fourteenth day the number of cell layers increases to twenty-four, as the type I structure is forming (Text-fig. 2; Pl. 3, figs. 17–19).

After the sixteenth day the full number of cell layers have appeared in the common carotid at the zone of bifurcation, and the whole structure resembles very closely the carotid arch at the level of origin of the subclavian, where the same number of cell layers are present with the same number of intimal and muscular layers. Intervening between these two points, however, is the whole length of the common carotid artery, of type III structure.

(8) *Persistent left fourth aortic arch in an* 11*-day embryo.* The left fourth aortic arch usually disappears during the seventh day of incubation, as described earlier in this paper, but in one particular 11-day embryo the left fourth aortic arch was still present, although with an open lumen at the proximal end only. Distally, the vessel gradually disappeared before reaching the aorta.

As, however, the proximal part showed considerable histological differentiation, and was re-

cognizable as a miniature artery of type I, a note describing this anomaly is given here (Pl. 2, fig. 15).

At its origin from the common aortic trunk, the left aortic arch was considerably smaller in diameter than the right arch, and the number of cell layers was one-third of that of the right arch. The inner three cell layers formed a recognizable intimal layer, and the remaining six were muscular layers with fine elastic layers between them. There were no non-muscular cells between the muscular layers. Some of the cells at the base of this arch were in mitosis and no pycnotic nuclei were seen. The endothelium was a definite cell layer surrounding a packed mass of blood corpuscles.

Distally the organized structure of the vessel wall was soon lost. First, the endothelium became disarranged, and blood corpuscles penetrated between the intimal layers. Then the lumen was lost, and finally also the layered condition of the cells. Only a strand of cells then remained with nuclei elongated in the direction of the strand, in between which ran fine elastic fibres. This strand ran through many sections, gradually containing fewer and fewer cells. Throughout its course nuclear degeneration was absent.

DISCUSSION

It is generally supposed that the development of the walls of blood vessels is influenced by the blood stream through the lumen. The primitive system of endothelial tubes is already in existence when the first circulation begins; the histological differentiation of the mesenchyme round the endothelium does not begin until later, when the blood has been circulating for some days and when the force of the blood stream is already much greater than when it first began. In succeeding stages, the blood stream becomes increasingly more powerful, and the walls of the main vessels become progressively more elaborate in structure. The analysis of the relations between these two sets of events is one of the most difficult problems which causal embryology has to solve.

At successive stages in vessel development, different aspects of the problem are predominant. First, the main question is the determination in the early mesenchyme of the cell types which will be found in the future vessel wall; later, when smooth muscle cells, elastic and collagen fibres have appeared, interest centres mainly on the question of what factors govern the amount of each tissue which is formed. The existing literature deals mainly with this second aspect of the problem, and is concerned with the influence of the blood stream on the thickness and structure of vessel walls. Thus, Thoma (1893) states his second histomechanical principle as follows: 'The growth in thickness of a vessel wall is proportional to the tension in the wall, which itself is determined by the diameter of the vessel lumen, and by the blood pressure.'

The evidence in favour of this hypothesis is derived entirely from the study of post-embryonic vessels. In general, arteries have thicker walls than veins, large arteries have thicker walls than small arteries, and where arteries increase in diameter under pathological conditions, the tunica media increases in thickness (Thoma, 1920). Furthermore, when segments of veins are transplanted into arteries, the wall of the transplant increases in thickness (Fischer, 1908). Finally, when an artery is ligated, and the circulation interrupted, the normal structure of the wall of the ligated artery is gradually lost (Schaeffer & Radasch, 1924).

Evidence derived from the study of adult material cannot, however, be applied directly to the development of the same structures in embryonic life. Although no structure in the adult body which performs a mechanical function is independent of the influence of that function on its structure, yet in the embryo, structures which will in adult life have a direct mechanical purpose are formed during development entirely independently of their future function. The development of isolated embryonic skeletal elements in tissue culture has provided striking illustrations of this fact (Fell, 1939).

In the vascular system, the primitive endothelial network is formed before the circulation begins, and therefore independently of it. The first differentiation of mesenchyme into endothelium is thus a self-differentiation. However, within a primary endothelial network main vessels can only be formed under the influence of the circulation through the network (Thoma, 1893), and once the circulation has acted on a network in this way, the calibre of the larger vessels thus formed is only maintained if the circulation continues. Thus the dynamic relationship between vessels and circulation characteristic of the adult circulatory system is early acquired in the embryo.

The wall of a fully developed vessel is made up of tension-bearing elements—extracellular elastic and collagen fibres, and smooth muscle fibres. The formation of all these in tissue cultures has been observed (Porta, 1930; Lewis, 1921), and several times the conclusion has been drawn that mechanical tension within the medium is necessary both for the formation and maintenance of tension-bearing elements. Doljanski & Roulet (1933, 1934) and Weiss (1934) have given a convincing picture of the way in which tensions are set up within the tissue culture medium. When a tissue is explanted containing already formed elastic or collagen fibres, these fibres degenerate (Porta, 1930; Möllendorff, 1932), which may best be explained on the grounds that when the tissue is isolated, tensions necessary for maintenance of the fibres are released. Applying this general line of reasoning to blood vessel de-

velopment, the mechanical tensions set up in the wall of a vessel by the passage of the blood stream through it are a necessary condition for the differentiation of tension bearing elements within the vessel wall.

The maintenance of these stresses in developing vessel walls is a function of the blood stream essential for continued development of the vessels. It may be that the performance of this function exhausts the influence which the blood stream exerts on developing vessel walls, and that the nature of the structure of a vessel wall is not determined by the type of mechanical stresses which it experiences during development, but by factors such as position and the influence of surrounding tissues and organs. On the other hand, the causal connexions between form and function in vessel development may be less limited than this, as Thoma suggests in his second principle.

Benninghof & Spanner (1929), in their study of the main vessels of an acardiac foetus, take the view that functional factors are fully capable of explaining the differences in the structure of different arteries. An acardiac foetus has a normal twin whose heart is responsible for the circulation in both. All the arteries of the acardiac twin are physiologically peripheral arteries of the normal twin. The most important result which was obtained by Benninghof & Spanner was that the dorsal aorta and common carotid arteries, which normally are elastic in type, in the acardiac foetus have the structure of peripheral muscular arteries. Their conclusion is expressed as follows: 'so entwickeln die Arterienwände nicht ortsgemäss sondern "kreislaufsgemäss", es kommt nur darauf an, welchen Abschnitt der Strombahn sie darstellen.'

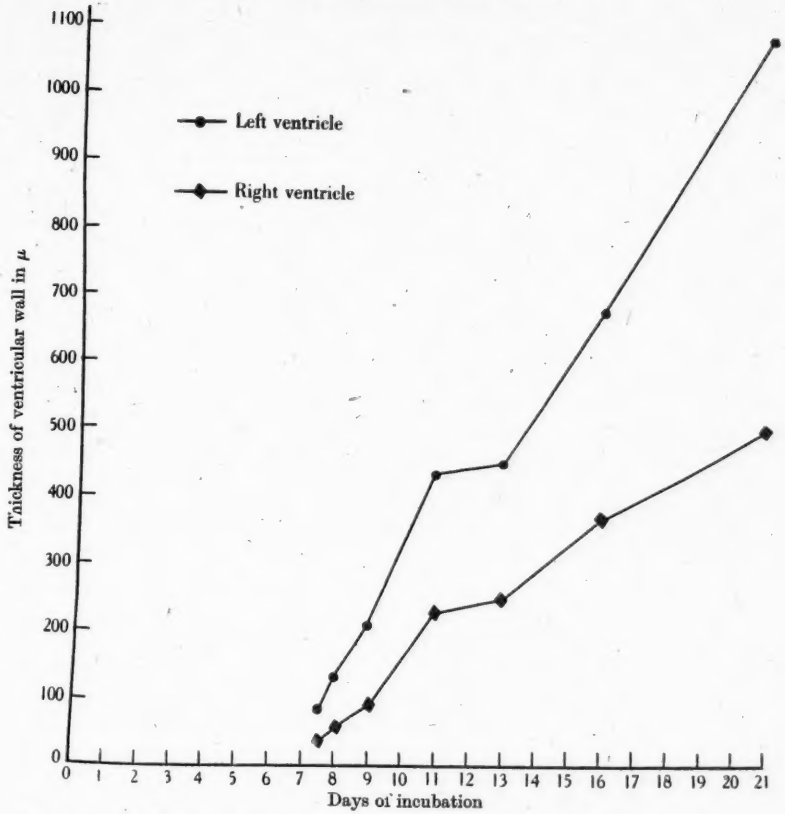
If this conclusion is true for all arteries, then all differences in structure between different arteries must be explicable on functional grounds. It is well known, however, that this is by no means so. The most striking example of difference in structure between adjacent arteries which defies a functional explanation is given by the ductus arteriosus. In mammals, the ductus is a muscular vessel, while both pulmonary and systemic arches are elastic, but in birds, as was first pointed out by Bremer (1924), the main difference between the ductus and the vessels immediately proximal and distal to it is the extreme thinness of that region of the ductus which closes first at hatching. Two other examples from the chick of abrupt discontinuity in structure along the course of a single artery may be given. One is the thickening of the wall of the common carotid just before its bifurcation into internal and external carotids, which extends through the region of bifurcation and rapidly gives place distally to the extreme thinness of wall of the cranial arteries, and secondly, the abrupt cessation of elastic fibres in the omphalo-mesenteric artery as it passes out beyond the body wall (Cohn & Lange, 1930). It is clear, therefore, that functional factors are not the sole determinants of arterial structure, and that these differences are causally related to factors outside the circulation, similar to those which control the self-differentiation of other systems of organs, yet for the operation of these extrinsic factors the existence of the circulation is still necessary in the way which has been outlined above.

On the other hand, differences in wall thickness between arteries of similar structure may in one instance in the chick embryo be related to differences in blood pressure within them.

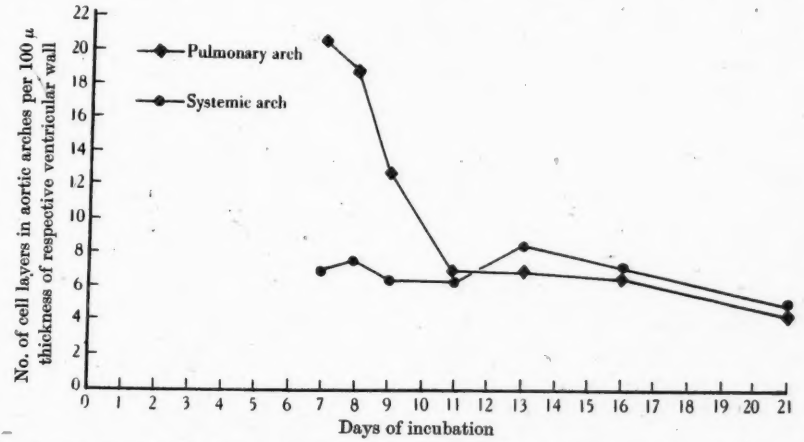
The aortic arches are a set of vessels of common structure and similar morphological position in which the extra-circulatory factors in development may be assumed to operate uniformly. There are considerable differences in wall thickness between the different arches, and these differences can largely be explained on functional grounds.

At the end of development, the arches which arise from the left ventricle have thicker walls than the pulmonary arches which arise from the right ventricle. The wall of the left ventricle at this time is more than twice as thick as the right ventricular wall, in measurements taken from the section series (Text-figs. 12, 13). It was shown by Woods (1892) in the adult human heart, that the thickness of the ventricular wall is proportional to the tension in the wall, as measured by the product of the intraventricular pressure and the wall curvature. Some indication that the same reasoning may be applied to the chick heart at the end of the incubation period is given by the fact that at the apex of the ventricles, where the curvature is greatest, the wall is thinner, both in the late chick embryo and in the adult. If, therefore, the thickness of the ventricular wall may be taken as a measure of the respective intraventricular pressure, the former may be compared with the thickness of the walls of the aortic arches which spring from that ventricle. Text-fig. 13 shows that from 11 days onwards the ratios of the wall thicknesses of the left ventricle and of the systemic arch, and of those of the right ventricle and the pulmonary arch, are in each case sufficiently regular to justify the conclusion that wall thickness in both ventricle and aortic arch is determined by the same agency.

The difference in thickness between the walls of left and right ventricles in the chick embryo is an interesting point of difference from the mammalian condition. The left and right ventricles of the mammalian foetus are of equal thickness, and the intraventricular pressures of the rabbit foetus have been found to be nearly equal (Hamilton, Woodbury & Woods, 1937). The difference between the anatomy of the mammalian foetal aortic arches and those of the chick embryo may perhaps explain why a pressure difference in the ventricles of the



Text-fig. 12. Thickness of ventricular walls of chick embryos from 7½ to 21 days of incubation.


Pulmonary arch
Systemic arch
No. of cell layers in aortic arches per 100 μ thickness of respective ventricular wall
Days of incubation

Text-fig. 13. Number of cell layers in the systemic arch per 100μ thickness of left ventricular wall, and of cell layers in the pulmonary arch per 100μ thickness of right ventricular wall.

embryo chick heart is possible without regurgitation of blood from the systemic to the pulmonary arches. The extreme length of these vessels, and the acute angle at which they meet, must hinder any backflow along the pulmonary arches.

In general we may say, therefore, that certain features in the development of the arterial walls point to their determination by haemodynamic factors, but that other features cannot be explained by reference to the circulation alone and must be related to position in the embryo and the influence of neighbouring structures. With only the present descriptive data in hand further analysis is not yet possible.

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EXPLANATION OF PLATES 1–4

PLATE 1

Fig. 1. Transverse section of 5-day embryo at the point of bifurcation of the aorta at the level indicated in the reconstruction of this section series in Text-fig. 1*a*. Stained: Wilder carmine. Notice well-developed argyrophil fibrils. ×293.

Fig. 2. Transverse section of 6½-day embryo at the point of bifurcation of the aorta at the level indicated in the reconstruction of this section series in Text-fig. 1*b*. Stained: Haematoxylin and eosin. Notice cell degeneration still present (*d*) with end stages predominating. ×530.

Fig. 3. Section through the aortic arch region of a 7-day embryo in a plane at right angles to the aortic arches, showing the degenerating remains of the left systemic arch. Stained: Haematoxylin and eosin. The left pulmonary and carotid arches are to the right and left of the photograph. ×200.

Fig. 4. Part of transverse section through the 7½-day embryo, of which the dorsal vessels are reconstructed in Text-fig. 1*c*. The section passes through the degenerating left ductus caroticus at the level shown in Text-fig. 1*c*. ×480.

Fig. 5. Section through the base of the aortic arch region of a 5½-day embryo, showing the earliest elastic fibres so far encountered. Stained: Weigert haematoxylin. Notice that the mesenchyme cells have not condensed round the endothelium of the carotid and systemic arches seen at the lower margin of the photograph, and that the elastin granules occur among mesenchyme cells some distance from the vessels. ×350.

Fig. 6. Section through the aortic arch region of a 7-day embryo, to show the development of elastic fibres at this stage. Stained: Weigert haematoxylin. Several layers of dense mesenchyme cells now surround the endothelium of the arches, as is seen in the pulmonary arch on the lower border of the photograph. The borders of these cells are outlined in elastin. In the loose mesenchyme beyond elastin granules are seen among the cells. ×312.

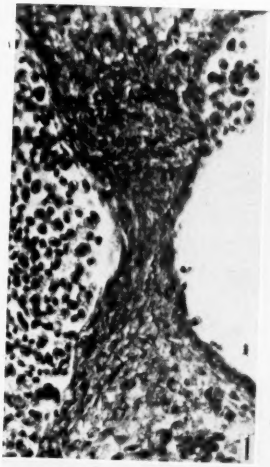
Fig. 7. Transverse section through the 6½-day embryo reconstructed in Text-fig. 1*b*, at the level indicated in the reconstruction, showing the left ductus caroticus at the top of the photograph and the left systemic arch beneath it. Stained: Haematoxylin eosin. Although the diameters of both vessels are very much reduced no cell degeneration has yet appeared. ×268.

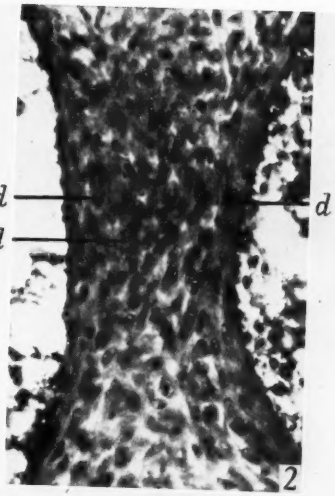
Fig. 8. Transverse section of a 7½-day embryo at the point of bifurcation of the aorta at the level indicated in the reconstruction of this section series in Text-fig. 1*c*. Stained: Weigert carmine. Notice that cell degeneration has ceased in this region and that elastic fibres have appeared. ×530.

Fig. 9. Part of the same section embryo as in fig. 8, in the region of the roots of the aortic arches. The left pulmonary arch is to the right of the photograph and the left carotid arch to the left. Showing the early stage in the differentiation of the aortic arch structure, and the greater development of elastic fibres in the pulmonary arch at this period. ×224.

PLATE 2

Fig. 10. Longitudinal section through the left pulmonary arch of a 13-day embryo. Stained: Orcein, Delafield. The lumen is to the right of the photograph. To show the arrangement of the muscle fibres of the tunica media in incomplete layers, which run longitudinally for limited distances. ×212.

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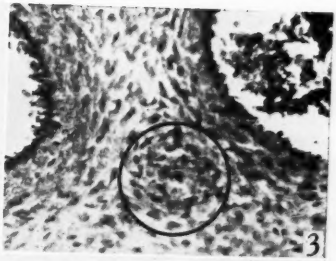

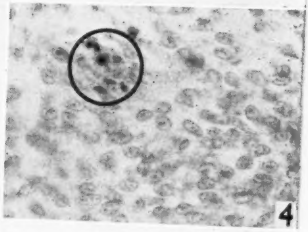

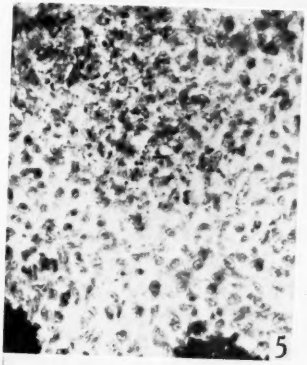

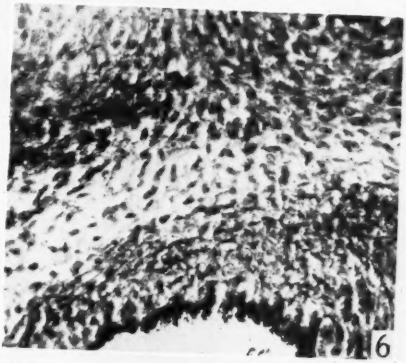

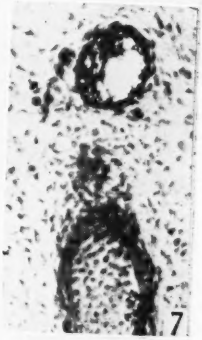

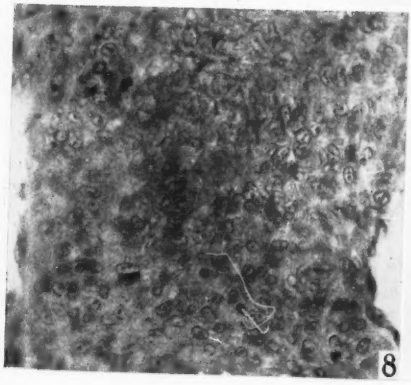

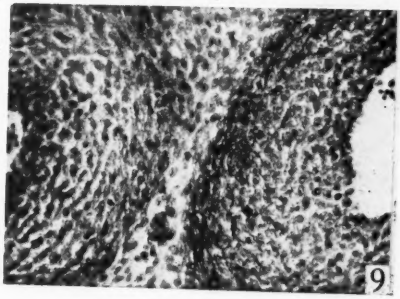

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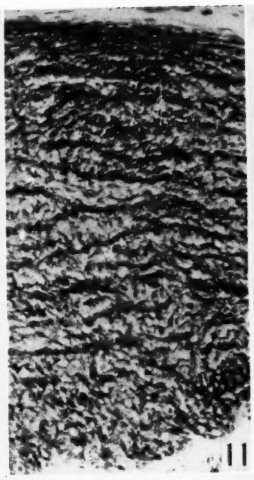
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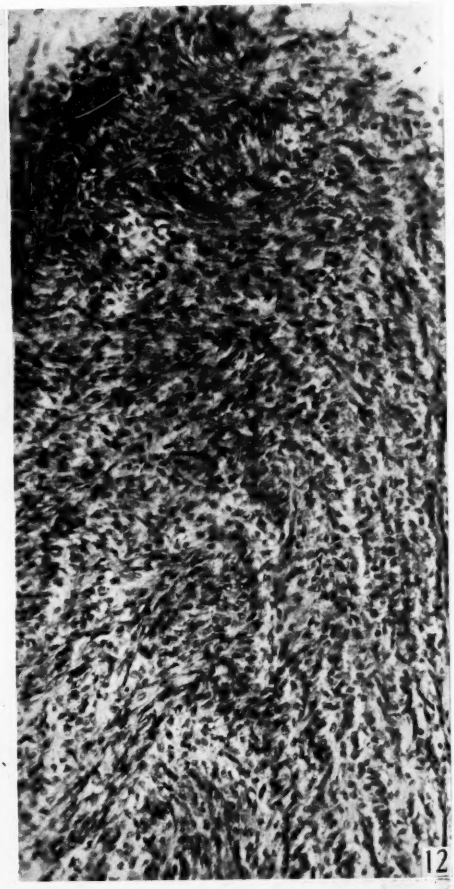
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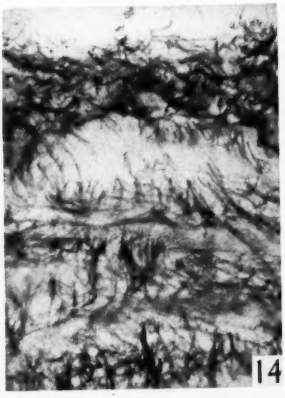
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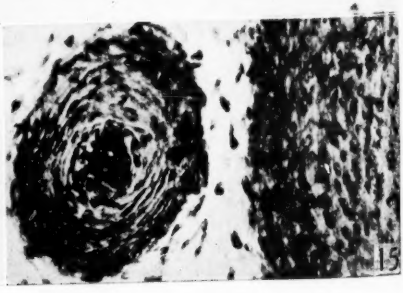
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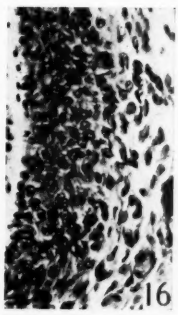
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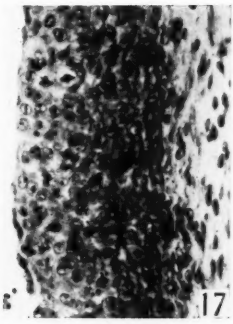
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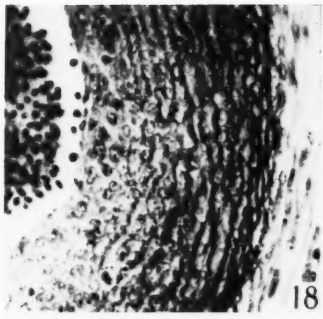
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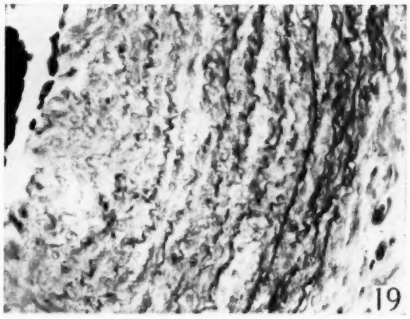
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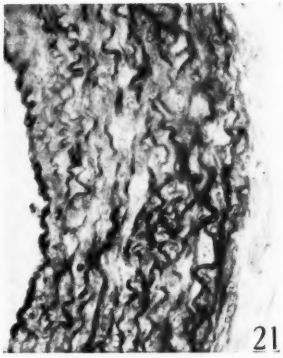
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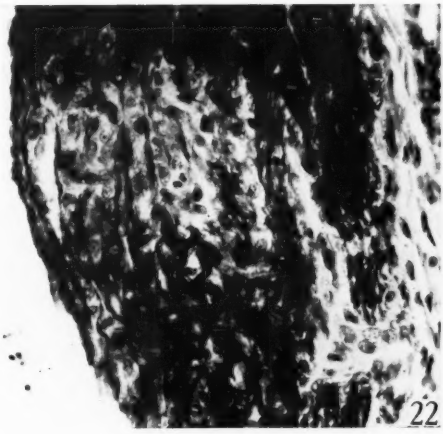
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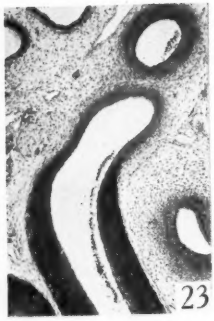
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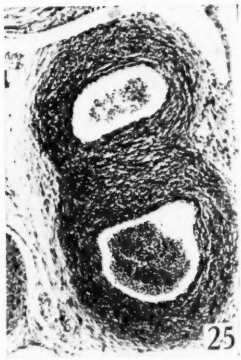
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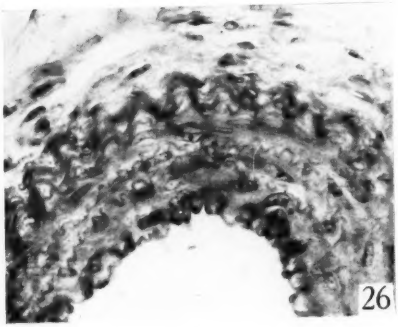
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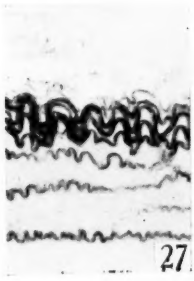
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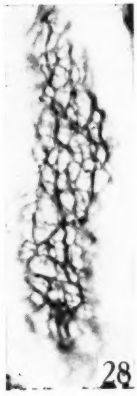
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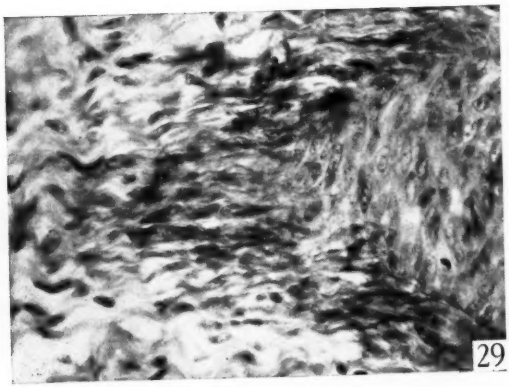
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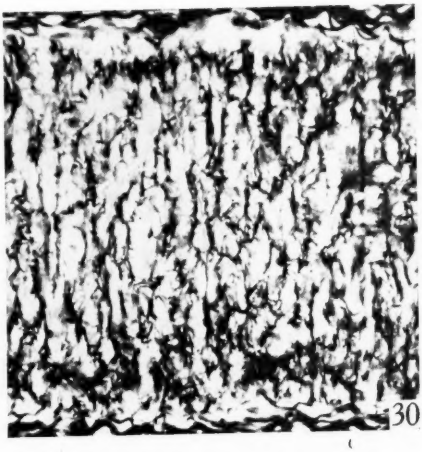
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Fig. 11. Transverse section through the systemic arch of a 20-day embryo. Stained: Orcein, Delafield. The lumen of the vessel is at the lower border of the photograph. The muscular layers appear as discontinuous arcs, separated by wide non-muscular layers through most of the thickness of the vessel wall. ×220.

Fig. 12. Oblique section through the wall of the right pulmonary arch of a 16-day embryo, to show the orientation of the muscle layers. Stained: Orcein, Delafield. The outer layers of the vessel wall are at the top of the photograph and the intimal layers are seen in the middle of the lower border. ×216.

Fig. 13. Longitudinal section through the right pulmonary arch of a 20-day embryo to show the arrangement of cells and elastic fibres in the intimal region. Stained: Orcein, Delafield. The lumen is on the right of the photograph. ×572.

Fig. 14. Oblique section through the systemic arch of a 1-day hatched chick, to show the arrangement of the elastic fibres in the outer layers of the vessel wall. Stained: Weigert picric. The outermost layer of the tunica media is at the top of the photograph. The muscle layers in this region run longitudinally and the elastic fibres which cover each side of the muscle layers are the short vertical fibres seen in the photograph. The elastic fibres on adjacent faces of one muscle layer and the next are joined by the horizontally running fibres. ×475.

Fig. 15. Part of transverse section through the aortic arch region of an 11-day embryo. Stained: Orcein, Delafield. Showing on the left the abnormally retained left fourth aortic arch, which has the structure of an immature normal arch. ×308.

PLATE 3

Fig. 16. Part of transverse section through the common carotid artery of a 10-day embryo, just proximal to the bifurcation. Stained: Weigert carmine. ×345.

Fig. 17. Part of transverse section through the common carotid artery in the same region of an 11-day embryo. Stained: Weigert carmine. ×385.

Fig. 18. Part of transverse section through the common carotid in the same region of a 12-day embryo. Stained: Weigert carmine. ×365.

Fig. 19. Part of transverse section through the common carotid in the same region of a 16-day embryo. Stained: Weigert carmine. ×336. Figs. 16–19 illustrate the rapid development of the distal type I carotid region.

Fig. 20. Part of transverse section through the same common carotid as in fig. 16, but more proximally in the transitional region between the main type III region and the region of the bifurcation. The non-muscular elastica externa is still present. Stained: Weigert carmine. ×336.

Fig. 21. Part of transverse section through the common carotid artery of a 1-day hatched chick in the region between subclavian and vertebral arteries where the structure is of type II. Stained: Orcein, Delafield. ×465.

Fig. 22. Part of transverse section through the systemic duct of a 20-day chick embryo to show the interruption of the elastic and muscular layers by glomal cells. Stained: Orcein, Delafield. ×430.

PLATE 4

Fig. 23. Part of transverse section through a 9-day embryo showing the abrupt change in passing from the pulmonary arch to the ductus arteriosus. The right systemic ductus is seen on the upper border of the photograph. Stained: Weigert carmine. ×23.

Fig. 24. Part of transverse section through a 13-day embryo just behind the pulmonary arches, showing the thin-walled proximal region of the ductus arteriosus (above), and the pulmonary artery below. Stained: Wilder azocarmine. ×80.

Fig. 25. Part of transverse section through the same 13-day embryo just before the fusion of the right ductus arteriosus (below) and systemic ductus (above). Both have walls of equal thickness and considerably thicker than that of the ductus arteriosus in the proximal region. Stained: Orcein, Delafield. ×80.

Fig. 26. Part of transverse section through the common carotid artery in the mid-neck region of a 1-day hatched chick, to illustrate the type III structure. Stained: Delafield. ×570.

Fig. 27. Part of transverse section through the same artery as in fig. 26 to show the arrangement of the elastic fibres. Stained: Weigert picric. ×560.

Fig. 28. Part of sagittal section through the pulmonary artery of a 1-day hatched chick, passing through the elastica interna, to illustrate its reticular nature. Stained: Weigert picric. ×650.

Fig. 29. Part of oblique section through the wall of the pulmonary artery of a 20-day chick embryo. The adventitia lies to the left of the photograph and the circular muscular zone of the tunica media to the right. Between them lies a region of longitudinal muscle and elastic fibres. Stained: Orcein, Delafield. ×530.

Fig. 30. Part of sagittal section through the pulmonary artery of the same chick embryo as in fig. 29 to show the reticular sheaths round the circular muscle fibres of the tunica media. Stained: Wilder. ×635.

THE REPRODUCTIVE SYSTEM OF A PREGNANT HERMAPHRODITE RABBIT (*ORYCTOLAGUS CUNICULUS*)

By E. M. SHEPPARD, *Department of Zoology and Comparative Anatomy, University College, Cardiff*

The hermaphrodite rabbit described in this paper was a wild animal of medium size, purchased for class purposes early in February; it was trapped in the Llandovery district of Carmarthenshire. So far as I am aware no case of hermaphroditism in the rabbit has ever been recorded, and amongst rodents this phenomenon is extremely rare. The present specimen was particularly interesting because separate ovaries and testes, the latter descended into scrotal sacs, were present on each side of the body; at the same time it was functioning as a normal female individual, as shown by the presence of two foetuses in the right uterus. In the majority of mammalian intersexes the genital system consists of a mixture of male and female organs, usually abnormal and non-functional, together with imperfect external genitalia; Brambell & Hall (1936), however, have described an intersexual lesser shrew (*Sorex minutus*) which functioned as a typical male individual, in spite of the fact that it had well-developed Fallopian tubes, uteri and vagina; no ovaries were present. This case is analogous to the present specimen except that in the latter the opposite sex was the functional one, and degenerate testes were present as well as ovaries.

The tissues of the reproductive system were not specially fixed for histological investigation as the abnormality of the specimen was not discovered until it had been partially dissected. For the same reason certain anatomical details unfortunately could not be verified.

OBSERVATIONS

The appearance of the external genitalia was interesting because it showed a very definite male trend in development on which that of the female was superimposed. The opening of the vestibule was large and rather elongated; the margins of the 'labia minora' projected and were considerably folded; the vulva, which normally is formed from the labio-scrotal folds, was not represented as such but was differentiated in the form of scrotal sacs similar to those of a normal male.

Lying in the ventral wall of the vestibule was an enlarged clitoris which projected beyond the margin of the opening, and ended in an epithelial 'tag' (Fig. 1 D; and cf. that of a normal female, Fig. 1 C). The opening of the vestibule was closed by the folding inwards of the labia and the bending upwards of the clitoris (Fig. 1 E); the latter was stiffened by strong corpora cavernosa and controlled by well-developed retractor muscles.

The ovaries, each attached to the dorsal wall of the abdomen by the mesovarium, were normal in position and appearance; no epoöphoron or paroöphoron could be found on either side of the body, but this was to be expected since these bodies represent the vestiges of the epididymis and paradidymis respectively, and remain in close association with the Wolffian duct and the testis. The differentiation of the Müllerian ducts was complete; normal Fallopian tubes, uteri, and vagina were present (Fig. 1 A), and two foetuses were present in the right uterus; there was a considerable accumulation of fat in the broad ligaments.

The male organs were much less complete; the testes, each covered with a tough tunica albuginea, were descended into scrotal sacs, to which they were attached by gubernacula in the typical manner. Each testis (Fig. 1 B) was of normal length, but much more flattened and less bulky than that of a typical male. The Wolffian ducts and their derivatives had presumably undergone a process of degeneration under the influence of the female sex hormone, for no vas deferens or epididymis could be found on either side; the uterus masculinus and prostate were also absent.

The vascular system of the reproductive organs was a combination of the male and female arrangement; for, in addition to the typical vessels of the female, there were spermatic arteries and veins, passing to and from the testes in the spermatic cords. The connexion of these vessels with those of the main system could not be traced as the cords had been broken before this examination was carried out.

A histological examination was made of the ovaries, uterus, vagina and testes; the sections were stained with Ehrlich's haematoxylin and eosin. Sections of testis were also counterstained with Van Gieson and picro-indigo-carmine, the former being used in order to get a picture comparable with that obtained by Crew & Fell (1922, pl. 18). Frozen sections of the testis were stained for fat with Sudan IV.

Ovaries. These appeared to be perfectly normal; Graafian follicles in various stages of development were present. Some of the larger follicles seemed to be undergoing a process of absorption but, according to Hammond & Marshall (1925, p. 80), follicular atrophy is quite common in the rabbit,

especially during periods of pregnancy and lactation.

In a section through the middle of the right ovary there were three corpora lutea, each of which was formed of trabeculae of large luteal cells which converged to a central connective tissue strand containing the small remaining portion of the central cavity; in other words, the corpora lutea were almost fully formed, showing that copulation must

membrane described by Chipman (1903), Hammond & Marshall (1925) and others, were well defined; the two mesometrical or placental folds were the largest and were well supplied with blood vessels. In the connective tissue between the uterine glands in both the peri- and ob-placental folds, though more frequently in the latter, were large solitary decidual cells, the cytoplasm of which was granular and deeply stained with eosin.

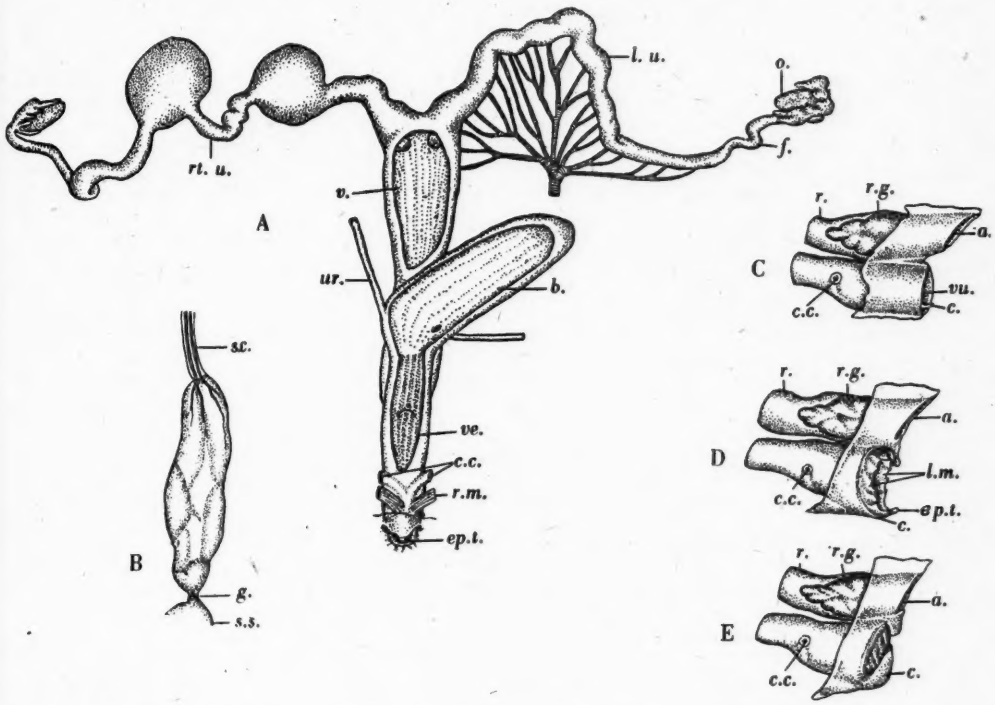

Fig. 1. A. Reproductive organs removed from the body and dissected from the ventral side. *o.* ovary, *f.* Fallopian tube, *rt.u.* and *l.u.* right and left uterus, *v.* vagina, *b.* bladder, *ve.* vestibule, *c.c.* corpora cavernosa, *r.m.* retractor muscle, *ur.* ureter, *ep.t.* epithelial tag. B. Testis withdrawn from its scrotal sac. *s.c.* spermatic cord, *g.* gubernaculum, *s.s.* remains of scrotal sac. C. Corresponding dissection of a normal female rabbit; the tip of the clitoris has been drawn outwards. *a.* anus, *r.* rectum, *r.g.* rectal gland, *vu.* vulva, *c.* clitoris, *c.c.* cut end of corpus cavernosum, *l.m.* labia minora. D and E. External genitalia seen from the left side after removal from the body. D. With the entrance to the vestibule open and E with it closed.

have taken place at least some ten days earlier. This assumption is based upon information obtained from statements made by Hammond & Marshall (1925, p. 61). A section of the left ovary showed the presence of a single corpus luteum undergoing atrophic changes.

Uterus. The wall of the uterus, as seen in transverse and longitudinal sections, was typical of that of a pregnant female rabbit; the muscular coat was hypertrophied and the six folds of the mucous

In the placental region the mucous membrane showed the structure typical of the normal pregnant uterus. The two blastocysts were not well preserved, and all that can be said about them is that they were firm and round, and measured about 20 mm. in breadth; they appeared to be undergoing normal development.

Vagina. The mucous membrane of the vagina was thrown into folds and its epithelial lining consisted of a single layer of columnar cells; the cyto-

plasm of these cells was vacuolated towards the base of the cell and granular towards the free surface; this appearance was very similar to that figured by Hammond & Marshall (1925, p. 90, pl. XI, fig. 3) for a pregnant rabbit with an accumulation of mucin at the base of the vaginal folds.

The result of this histological examination of the female organs of the hermaphrodite showed that the tissues did not differ from those of a typical female rabbit.

*Testis.** The structure of the testis was degenerate, but small lobules of seminiferous tubules, separated from each other by areas of adipose tissue, were discernible. The tubules were most abundant in the deeper regions around the blood vessels, while the peripheral areas consisted almost completely of fat separated into lobes by strands of fibrous connective tissue. A fairly large lymph nodule was present near the base of the testis.

The seminiferous tubules were much reduced in size, compressed and irregular in outline. White fibrous tissue, the elongated nuclei of which were clearly visible, was present between the basement membrane and the lining epithelium. Such a layer of tissue was observed by Crew & Fell (1922, p. 567) in the undescended testis of a rabbit; they suggested that it was similar to the intertubular fibrous tissue and was presumably formed by the cells of the basement membrane.

The tubules were not all in the same stage of degeneration, and some of the stages, mentioned by Crew & Fell in their paper quoted above, could be recognized; in no tubule was there any sign of active spermatogenesis and it seems unlikely that any had ever taken place.

In the less degenerate tubules the epithelium was represented by a single, often incomplete, layer of cells, the nuclei of which were quite distinct but the cytoplasm was fragmentary. Two types of cells, differing in the form of their nuclei, could be recognized; these, interpreted according to Crew & Fell, represented (1) cells of Sertoli with small ovoid and rather granular nuclei, and (2) spermatogonia in which the nuclei were larger and in many cases showed signs of degeneration; this was indicated by increased size, and the peripheral displacement of the chromatin in the form of granules.

Other tubules were more degenerate; in these most of the cells had broken away from the basement membrane and the lumina contained masses of cell detritus. In some of the tubules one or more spherical bodies, the outlines of which were clearly defined, were present in the cell debris; each consisted of a degenerate nucleus surrounded by a concentric layer of vacuolated cytoplasm and resembled the ovum-like 'intra-tubular body' of Crew & Fell (1922, pp. 561–2 in the goat, and p. 569 in the rabbit). According to these authors the 'intra-tubular body' is produced as the result of colloid degeneration, either by the liquefaction of cell detritus around a degenerating nucleus or by the coalescence of several small 'colloid globules' in the midst of cell debris. The bodies were not as large or as deeply stained as those described by Crew & Fell, neither did they show any sign of calcification; it is probable that they represented an earlier stage in formation.

The intertubular tissue consisted almost entirely of metamorphosed interstitial cells, separated into lobules by strands of fibrous connective tissue. These cells appeared in one of three forms: (*a*) cells of oval or rounded shape, the outlines of which were very distinct, with granular cytoplasm and rounded, rather deeply stained, nuclei; these cells were present in the regions near the tubules and were few in number; (*b*) cells which were rather diffuse and vacuolated, with larger, less deeply stained, nuclei in which the chromatin appeared in the form of a reticulum; and (*c*) large cells almost entirely fitted with lipoids (as shown by the Sudan IV preparations), and in which the nuclei were small and eccentrically placed.

According to Crew & Fell (1922), who describe similar types of interstitial cells in a number of ectopic testes, these cells are not 'fat forming' but are hypertrophied by the accumulation within them of various fat globules and lipoids representing stored food material which normally would be used by the germ cells during spermatogenesis.

DISCUSSION

Hermaphroditism in certain mammals, especially in certain strains of pigs, is known to be fairly common; a number of cases has been described by various workers (Baker, 1925 *a*, *b*, 1926, 1928; Brambell, 1929; Crew, 1924; Pick, 1914). Amongst rodents, however, the abnormality is extremely rare, and, as Brambell (1930, p. 157) points out, no case in the mouse or rat is known, in spite of the fact that they are used in large quantities for laboratory work. Hermaphroditism in the rabbit has not been recorded previously although this animal is extensively used as a dissection type for teaching purposes.

The particular interest of the specimen described in this paper is not so much that it is unique amongst recorded abnormal rabbits in exhibiting hermaphroditism, but that it belongs to the rarest group of hermaphrodite types, namely, that in which a separate ovary and testis are present on each side of the body in their normal positions; the only other example of such a condition was

* The sections of this tissue were submitted to Prof. F. W. R. Brambell for confirmatory examination, and he has expressed his opinion that it is probably testicular and not mesonephric.

described by Walentowicz (1888), in a pig. Furthermore, the rabbit was pregnant and so was functioning as a normal female.

According to Brambell (1930, p. 163) and others, all vertebrates have the potentialities of both sexes during embryological development, but the decisive factor which actually determines the final development of the sex of the individual is the sex chromosome. The reproductive system in early development consists of paired indifferent gonads which, nevertheless, will have the chromosomal constitution of either the male or the female sex; at this stage the rudiments of both the Wolffian and the Müllerian ducts are present and the external genitalia are represented by the genital tubercle, etc. From this condition, which is exhibited by both sexes, either the male or the female type becomes organized.

It is well known that, in the development of the reproductive system, the gonad becomes differentiated first, then the external genitalia and lastly the ducts. Since the sex hormone is produced by the differentiating gonad, the external genitalia and the ducts will develop under its influence (male or female, as the case may be). It is also known that in mammals the differentiation of the gonad from the genital ridge commences with the proliferation of medullary cords in the cephalic part of the ridge and this finally extends along its entire length.

In man, and presumably in mammals generally, the medullary cords of the cephalic region of each ridge degenerate and the adult testis is formed from the caudal portion, the medullary cords of which give rise to the testicular tissue; at the same time, the connective tissue capsule thickens to form the tunica albuginea of the differentiated testis. The development of the ovary from the indifferent gonad takes place slightly later in time, from a second proliferation of the germinal epithelium which gives rise to the cortical cords. These grow inwards and replace the medullary cords which undergo retrogressive changes. In the male the production of the sex hormone by the testis results in the development of the penis and of the scrotal sacs, and the further growth and differentiation of the Wolffian ducts; at the same time it inhibits any further alteration in the Müllerian ducts which soon atrophy. In the female the reverse takes place, the sex hormone from the ovaries leads to the formation of the vulva and clitoris, the growth and differentiation of the Müllerian ducts, and the cessation of further changes in the Wolffian ducts which almost completely degenerate. Brambell (1930) and Crew (1924) both came to the conclusion that Goldschmidt's hypothesis can be applied to all known cases of mammalian intersexuality; this is based on his observations on intersexuality, resulting from certain racial crosses, exhibited by the offspring of the gipsy moth (*Lymantria dispar*). The hypothesis involves three fundamental ideas which are expressed very briefly by Brambell (1930, p. 651) as: '(1) The Mendelian factors determining sex are quantitative. (2) Racial variations occur in them. (3) A time factor is involved in their expression during development.' In the genotypic male therefore, during the developmental changes in the genital ridge, the male-differentiating substances will be in excess of the female-differentiating substances; in the genotypic female the opposite will be the case. If, for any reason, during the development of the reproductive system the action of the differentiating substances of the genotypic sex is delayed, then that of the opposite sex may produce some effect on the indifferent gonad before its action is overridden by that of the genotypic sex, and an intersexual individual will result. The amount of intersexuality exhibited will depend upon the time at which the overriding occurs.

Crew (1927, p. 95) explains this by assuming '(1) that in these cases, males genetically, the sex-determining gene-complex included "quickly elaborating" female-determining genes and "slowly elaborating" male-determining genes; and (2) that the differentiation of the gonads is not synchronous but consecutive, the left being affected before the right, and the cephalad pole before the caudad.' Crew considers that intersexuality usually occurs in the genetic male and points out that this is not surprising since the male is the digametic (*XO*) sex, and the sex complex is less stable in the digametic sex than in the monogametic (*XX*). Nevertheless, he agrees that there is evidence to show that intersexuality can occur in the genetic female, in which case the female-determining genes will be the 'slowly elaborating' genes, and the order of differentiation of the gonads will be from right to left, and from caudad to cephalad.

The present specimen can best be interpreted as a genotypic female in which the sex-determining gene complex included 'quickly elaborating' male genes and 'slowly elaborating' female genes, with the result that the minimum stimulus for gonad differentiation was reached by the male-determining genes first, and the caudal part of each genital ridge was laid down as a testis; thus differentiation started under the influence of the male-determining genes and resulted in the formation of testicular tissue and the consequent production of the male sex hormone: this sex hormone influenced the early differentiation of the external genitalia so that the urethral groove was of the male type.

At this stage the more 'slowly elaborating' female-determining genes exerted their influence and the ovaries became differentiated by the development of cortical cords produced by a second proliferation of the germinal epithelium (Fig. 2).

The formation of these cords started at the cephalic end of each genital ridge where, presumably, they replaced the already degenerating medullary

cords and extended backwards to the region occupied by the testis; here they were unable to penetrate the thickened tunica albuginea and proliferation ceased. Thus the stimulus of the female sex hormone, derived from the differentiating ovaries, was so much delayed that it was unable to affect the development of the caudal part of each ridge.

Measurements of the ovary, compared with those of a normal female, showed that the breadth was the same in both cases but that the ovary of the hermaphrodite was slightly shorter (10 mm. as compared with 12 mm.); this suggests that the proliferation of the cortical cords ceased sooner than it would have done in a normal female.

How much of the genital ridge is involved in the production of the normal adult ovary is uncertain, but in all cases of mammalian intersexuality in which an ovotestis is described, the ovarian tissue forms a cap over the anterior end of the testis; it is of interest to note that in the female mole, *Talpa europaea*, referred to below, the ovary is divided into two parts, the anterior half of which is the functional ovary and the posterior part forms the so-called 'interstitial gland' which is said to be homologous with the testis.

These facts indicate the possibility that the ovary normally may be formed from the more anterior portion of the ridge, but this is masked by the speed at which differentiation occurs.

The influence of the female sex hormone of the hermaphrodite, overriding that of the male, resulted in the cessation of the male influence on the further development of the external genitalia and of the ducts which, owing to their normally later differentiation, were least affected by the male sex hormone. The effect on the external genitalia was to produce: (1) an intersexual condition in which the lips of the slit-like urinogenital opening failed to fuse (hypospadias) as they would have done had the male influence persisted; (2) an enlarged clitoris ending in an epithelial 'tag'; (3) abnormally prominent 'labia minora' (urethral folds of the in-

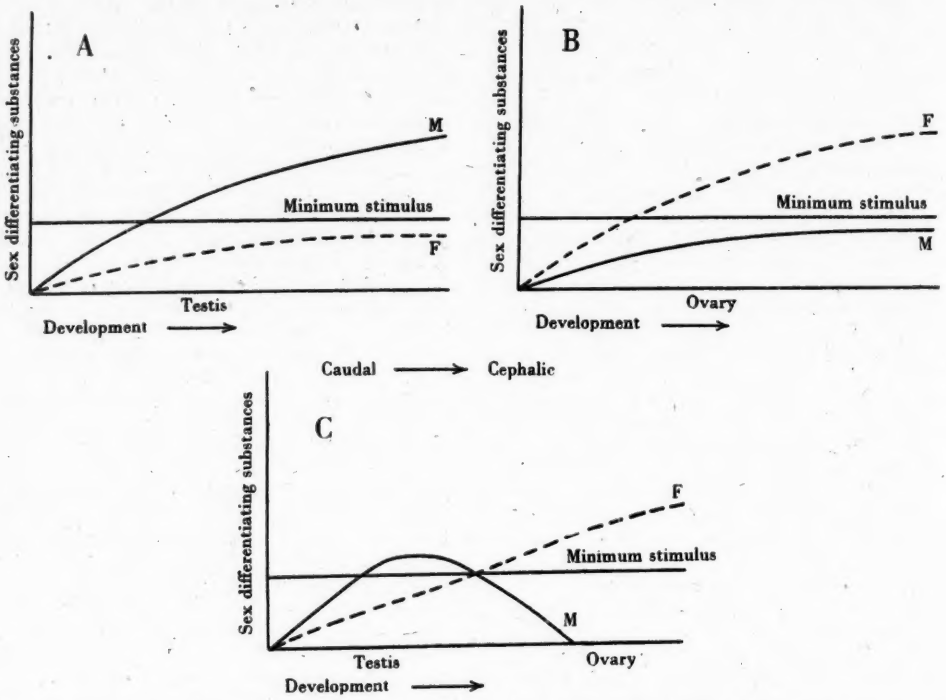

Fig. 2. Graphic representation of gonad differentiation. A. Normal genotypic male (after Crew) (male differentiating substances in excess throughout the period of gonad differentiation). B. Normal genotypic female (after Crew) (female differentiating substances in excess throughout the period of differentiation). C. Hermaphrodite rabbit. 'Quickly elaborating' male differentiating substances producing testes, later overridden by 'slowly elaborating' female differentiating substances producing ovaries. *M*, male-differentiating, and *F*, female-differentiating substances.

different stage); and (4) the absence of a normal vulva, since the labio-scrotal folds had already differentiated as the scrotal sacs.

The differentiation of the ducts must have proceeded completely under the influence of the female sex hormone, since the Müllerian ducts pursued their normal course in development, differentiating into Fallopian tubes, uteri, vagina and vestibule, whereas the Wolffian ducts and their derivatives, if they had begun to differentiate, had undergone a process of atrophy.

In this connexion it is interesting to note that the female mole, according to Matthews (1935), normally exhibits a form of intersexuality which he explains in the same way as the above. This animal is exceptional in having a male type of external genitalia, the clitoris is long and peniform, and the utero-vaginal canal has only an outlet to the exterior during the breeding season. The ovary is made up of two parts, an anterior functional ovary and a posterior so-called interstitial gland; these two parts undergo alternating periods of activity; the interstitial gland is regarded as homologous with the testis of the male, but it has no corresponding ducts. Matthews considers the female mole as a glandular hermaphrodite or female intersex during the period of anoestrus (when the interstitial gland is active), and as a functional female during the breeding season.

SUMMARY

1. A case of a pregnant hermaphrodite rabbit is recorded for the first time; there were a separate ovary and testis (the latter in a scrotal sac) on each side of the body.

2. The female organs were typical of those of a normal female, except for the external genitalia which were of an intersexual type; the male organs were represented by degenerate testes only.

3. Histological examination of the tissues of the reproductive system showed that the female organs were quite normal, but that the testes were undergoing fatty degeneration.

4. The type of intersexuality exhibited by the hermaphrodite is that of a genotypic female, in which the sex-determining gene complex includes 'quickly elaborating' male-determining genes, and 'slowly elaborating' female-determining genes.

I wish to record my thanks to Prof. F. W. R. Brambell, D.Sc., for examining some of my preparations.

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SPECIALIZED CONDUCTING TISSUE IN THE HEART OF THE COMMON HEDGEHOG (*ERINACEUS EUROPAEUS*)

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The present investigation was undertaken in order that a complete account of the specialized conducting tissue in the heart of an insectivore might be made available.

The detailed distribution of the specialized conducting tissue in the heart of the common hedgehog (*Erinaceus europaeus*) has not hitherto been described. Holmes (1921) gave a very brief description of the atrio-ventricular node and bundle in this animal. He described the node as being very similar to that of the sheep and stated that the bundle could be distinguished from the node by the appearance in the former of Purkinje fibres with very small compartments, but that the limbs of the bundle could only be identified by tracing serially their connexion with the main bundle.

Technique. Similar methods of preparation were used as described in a previous paper for the golden hamster (Walls, 1942).

GENERAL DESCRIPTION OF HEART

Features of interest in the gross anatomy of the hedgehog's heart are these:

(1) The opening of the inferior vena cava into the right atrium is guarded by right and left venous valves and that of the coronary sinus by a right valve only. The substance of these valves is in great part muscular (Pl. 1, fig. 5).

(2) The inter-atrial septum about half-way down its extent is joined to the ventral wall of the atria only by a very thin layer of fibrous tissue, the two atria at this place being very nearly in communication (Pl. 1, fig. 3; Pl. 2, fig. 11).

(3) In the base of the right venous valve in one of the hearts examined there were two small nodules of cartilage (Pl. 1, fig. 2). In all the hearts which were sectioned cartilage occurs to a greater or lesser extent in the fibrous skeleton of the heart base.

(4) A recess from the posterior wall of the aorta arises close to the main mass of the atrio-ventricular node (Pl. 2, fig. 10). In position and character it is almost identical with a similar structure in the hamster (Walls, 1942).

(5) Between the left venous valve and the inter-atrial septum there is a well-marked interseptovalvular space (Pl. 1, fig. 1).

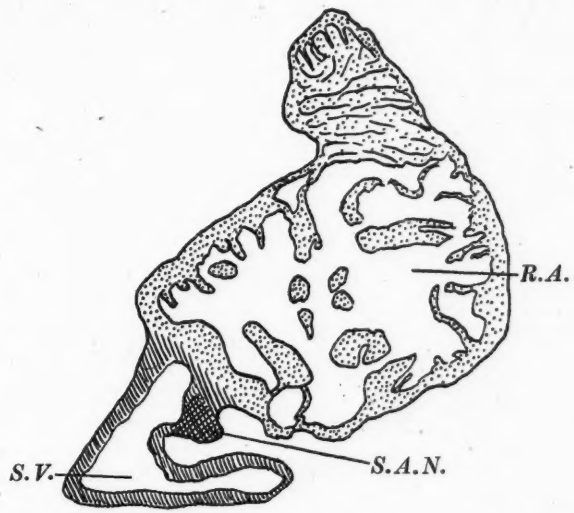
The heart rate, determined electrically, is 300 per minute.

Specialized tissue in the heart

The sinu-atrial node. This structure is found at the entrance of the superior vena cava to the right atrium in relation to the upper part of the sulcus terminalis (Text-fig. 1). It is crescentic in shape, the posterior horn of the crescent reaching a short distance down the sulcus, the remainder of the node being related to the roof of the atrium close to the superior caval opening. The node, which extends through the entire thickness of the heart wall from epicardium to endocardium, is not so extensive as that previously described in other mammals, lacking the tapering extremities shown by Davies (1942) in his figure representing the mammalian node. Running through the node is the nodal artery (Pl. 2, fig. 7) as observed by several authors in the mammal and by Davies (1930) in the bird. The fibres of the sinu-atrial node are completely cross-striated. This shows well with silver impregnation (Pl. 2, fig. 8) but is less obvious with other stains. In size the nodal fibres average about 6·5μ compared with 8μ for ordinary atrial fibres. They are fusiform in shape and show true branching, establishing continuity with each other and also with the adjacent atrial and sinus muscle. The junction effected by the nodal fibres with the atrial and sinus muscle fibres is direct and quite complete, no evidence being found of any septum between the two types of fibre. No connexion could be found between the sinu-atrial node and the atrio-ventricular node other than ordinary atrial muscle fibres. The posterior horn of the sinu-atrial node does not extend as far down the sulcus terminalis as the right venous valve guarding the opening of the inferior vena cava. It is clear, however, that it is related only to the right side and roof of the atrium and does not extend over to the left side in line with the more caudally placed left sinus valve. This is important. In his paper on the monotreme heart Davies (1931) writes as follows: 'It is commonly thought that the limitation of specialized nodal tissue in the higher mammalian heart to the base of the right sinus valve represents a reduction of the sinu-auricular ring of the lower reptile and the fish, and it is suggested by Mackenzie (1913) and by Walmsley (1929) that this reduction occurs *pari passu* with, and is caused by, the fusion of the left sinus valve with the inter-auricular septum, with consequent obliteration of the interseptovalvular space and disappearance of the left part of the sinu-auricular ring. But, as described above,

in the monotreme heart the sinu-auricular node is confined to the region of the right sinus valve (and crista terminalis) and the intersepto-valvular space is a well-defined region of the right auricle, between the left sinus valve and the inter-auricular septum. The obliteration of the intersepto-valvular space which occurs in the hearts of higher mammals cannot therefore be the prime factor in the reduction of the sinu-auricular ring of the lower vertebrate heart.' The occurrence of a condition in the hedgehog heart similar to that found in the monotreme adds weight to this argument.

The atrio-ventricular node. This structure is found in the lower part of the inter-atrial septum in front of the opening of the coronary sinus (Pl. 2, fig. 10).



Text-fig. 1. Outline (simplified) of a transverse section through the upper part of the right atrium to show the position of the sinu-atrial node. *S.V.* sinus venosus; *S.A.N.* sinu-atrial node; *R.A.* right atrium.

It forms a mass of loosely arranged, freely branching muscle fibres with which is mixed a little fibrous tissue. The node is more vascular than the neighbouring atrial muscle. Its fibres, which are transversely striated, spindle-shaped and branched, average 5–6μ in size and become directly continuous with both right and left atrial musculature. Projecting back into the node from the posterior surface of the aorta is the recess of the aortic wall already described. To the wall of this recess some of the nodal fibres are attached, and indeed a number of the fibres of the node which join with the left atrial muscle fibres do so by passing to the left through the substance of the recess wall. What purpose is served by the above arrangement it is difficult to say, but that it may form the anatomical basis for an additional, and hitherto undescribed, mechanism for controlling cardiac rate or rhythm associated with changes in aortic pressure is an attractive possibility.

The atrio-ventricular bundle. The bundle arises from the lower end of the atrio-ventricular node and passes to the right immediately behind the aorta. It is surrounded by a fine fibrous tissue sheath. After a short course in which it passes deep to the septal cusp of the tricuspid valve, it divides over the fleshy inter-ventricular septum into right and left limbs (Pl. 2, fig. 9). No branches from the bundle or its limbs go into any of the cusps of the tricuspid valve as in the bird (Davies, 1930) and hamster (Walls, 1942). Nor are there any early descending branches arising from the bundle prior to its division as in the rabbit (Lloyd, 1930). The atrio-ventricular bundle is the sole connexion between atria and ventricles in the hedgehog. Kent (1893) stated that in the hedgehog part of the auricular muscle sweeps uninterruptedly into the ventricle. I was unable to find any muscular connexion, however slight, between the atria and the ventricles other than the atrio-ventricular bundle. In certain sections the appearance at first glance does suggest that muscle fibres from the atria are continuing without break into the ventricles, but in all cases careful examination of serial sections proves convincingly that such appearances are illusory and are due to the obliquity of the plane of section.

On detailed examination the atrio-ventricular bundle is seen to be composed of muscle fibres em-

bedded in fibrous tissue. The fibres, which are only faintly striated, are from 6 to 7μ in breadth, being in many cases smaller than the neighbouring ventricular fibres which range from 7 to 10μ in diameter. It is to be emphasized that in no way do the fibres of the bundle approach in appearance or size to typical Purkinje fibres.

The limbs of the bundle. Holmes (1921) said that he could only identify the limbs by tracing their connexion with the main atrio-ventricular bundle in serial sections. This is true as far as tracing the uppermost parts of the limbs is concerned—a condition of affairs very similar to what is found in the human heart (Blair & Davies, 1935; Evans, 1938)—but after a short course large typical Purkinje fibres do appear in both limbs. In Pl. 2, fig. 12, Purkinje fibres of the left limb of the bundle are shown cut in transverse section, and in Pl. 3, fig. 13, similar fibres of the right limb are seen cut in longitudinal section. In each case these fibres are subendocardial in position, show transverse striation and average 25μ in breadth. The presence of Purkinje fibres in the limbs of the bundle does not continue for more than a third of the way down the inter-ventricular septum; thereafter the fibres of the limbs disappear by becoming continuous with the adjacent myocardial fibres. However, about half-way down the septum a few large typical Purkinje fibres are found buried deep in its substance. They become continuous with the adjacent ventricular fibres, but neither above nor below do they effect union with other than structurally undifferentiated cardiac muscle fibres. Points of difference between these 'isolated' Purkinje fibres and the Purkinje fibres of the limbs are their lack of transverse striation (Pl. 3, fig. 14) and their smaller size, being on average 15μ in cross-section.

Crossing the cavity of the right ventricle are several strands of muscle fibres, one of which, much larger than the others, is presumably the moderator band. These were examined carefully but no fibres other than ordinary cardiac muscle fibres were found.

Examination of the slender false tendons of the left ventricle also gave negative results.

Blood supply to the conducting tissue. The sinu-atrial node is traversed by a comparatively large artery, but whether this is a branch of the right or left coronary artery could not be established. In any event, the presence of this vessel in the node forms a striking contrast to the relative avascularity of the neighbouring heart wall (Pl. 2, fig. 7). The atrio-ventricular node also is well supplied with blood, the source being clearly the right coronary artery. In Pl. 2, fig. 10, the nodal branch from that vessel can be seen running back round the aorta to reach the node.

The atrio-ventricular bundle also is supplied by the right coronary artery, but no special branches of supply to the limbs of the bundle could be found. The inter-ventricular septum itself is so well supplied by both coronary arteries that definitive branches of supply to the limbs of the bundle are unnecessary. The left coronary artery is considerably larger than the right, the left ventricle wall being very thick—in places as much as five times the thickness of the free wall of the right ventricle: this great disparity in thickness was noted also in the case of the hamster (Walls, 1942) in which animal the left ventricle is, over most of its extent, six times as thick as that of the right.

Nerve cells and nerve fibres in relation to the specialized tissue. All the nerve cells in the heart of *Erinaceus europaeus*, whether occurring singly or in aggregations to form ganglia, are found on the atrial side of the coronary sulcus. This arrangement is similar to what obtains in man (Blair & Davies, 1935, and various earlier workers cited by these authors), the dog, cat, rabbit and guinea-pig (Woollard, 1926), the rat (Meiklejohn, 1913) and the hamster (Walls, 1942): but it is different from what is found in the ox. In the ox, nerve cells are present in both limbs of the atrio-ventricular bundle (Wilson, 1909; Blair & Davies, 1935), and indeed the latter authors described the presence of nerve cells in the epicardium more than half-way down the ventricles in the heart of the calf.

In the hedgehog the largest cardiac ganglion is found in the upper part of the inter-atrial septum and from it fibres pass to both nodes. Smaller ganglia occur in the anterior wall of the upper part of the right atrium, in the anterior and left walls of the left atrium, between the aorta and pulmonary artery, and around the coronary sinus. No nerve cells are found within the sinu-atrial node or atrio-ventricular node, or in the atrio-ventricular bundle.

The nerve cells in the cardiac ganglia are all of one type, large and round with a finely granular cytoplasm (Pl. 3, fig. 16). No cells corresponding to the second ganglion cell type of Kiss (1932) are found. In association with the nerve cells in the ganglia there are clumps of small dark cells (Pl. 3, fig. 17) which in size and general appearance are very similar to small lymphocytes.

Numerous nerve fibres are demonstrable in the substance of the sinu-atrial node (Pl. 3, fig. 15). The fibres are finely myelinated and show little oval swellings along their length. From these swellings non-myelinated fibres arise and pass to the nodal fibres around which they terminate without further structural specialization.

Nerve fibres are less numerous in the atrio-ventricular node and in the atrio-ventricular bundle still fewer; the nerve endings in each of these structures are of simple filamentous type, the parent fibre being non-myelinated along its whole length.

DISCUSSION

In the heart of the common hedgehog (*Erinaceus europaeus*) all the various collections of specialized tissue which comprise the mammalian cardiac conducting system can be recognized. There are, however, several features of interest in the distribution of this tissue. The sinu-atrial node, which is limited to the right side and roof of the sinu-atrial junction, despite the presence of a well-marked intersepto-valvular space, is not connected with the atrio-ventricular node by any tissue other than unspecialized atrial muscle fibres. Moreover, no Purkinje tissue occurs anywhere in either atrium. This is interesting, bearing in mind the heart rate of 300 per min. In the rapidly beating bird's heart a subendocardial Purkinje network as well as penetrating Purkinje fibres are found (Davies, 1930), being needed for the rapid transmission of the cardiac impulse which may occur as frequently as 1000 times per min. (canary). In the hamster (Walls, 1942) Purkinje fibres are present in the sinu-atrial node but not elsewhere in the atria, the heart rate in that animal being 450 per min. In the hedgehog, where the heart rate has fallen to 300, no Purkinje fibres occur in the atria.

Another interesting feature is that the atrio-ventricular valves are non-muscular and consequently no slips from the atrio-ventricular node or bundle are found to enter them. Such slips are present in the avian heart and hamster heart in which the muscular valves presumably contract actively in order that proper synchronization of the cardiac cycle may result. The absence of muscular tissue from the atrio-ventricular valves in the hedgehog heart suggests that valvular inertia does not impede a heart beating at 300 per min.

The last feature of special importance is the relative paucity of Purkinje tissue in the ventricles. In the proximal parts of both limbs of the bundle large typical Purkinje fibres are numerous. They lie in the subendocardium but disappear beyond the upper third of the inter-ventricular septum. No other structurally specialized fibres occur in the ventricles except for a few Purkinje fibres in the depths of the septum about half-way down. These few fibres are the only penetrating Purkinje fibres in the entire heart.

It will be seen therefore, that, in the main, the specialized cardiac conducting tissue in the hedgehog follows the general pattern for mammals.

SUMMARY

1. The specialized conducting tissue in the heart of the common hedgehog is described.

2. The limitation of the sinu-atrial node to the right side of the sinu-atrial junction is noted despite the presence of an intersepto-valvular space.

3. The sinu-atrial node and atrio-ventricular node are not connected directly by any strand of specialized tissue.

4. Nerve elements in relation to the specialized tissue are described.

5. Certain features in the gross anatomy of the heart which are of special interest are described.

I wish to thank Prof. C. M. West for helpful criticism, and Mr A. Welch for technical assistance.

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EXPLANATION OF PLATES

All figures are untouched photomicrographs. All the sections photographed were prepared by silver impregnation except those from which Figs. 1, 7, 10 and 11 were taken. These are of sections stained with haemalum and eosin.

Plate 1

Fig. 1. Transverse section through both atria to show venous valves and intersepto-valvular space. × 12.

Fig. 2. Right venous valve. In the base of the valve several nodules of cartilage can be seen. × 80.

Fig. 3. Transverse section through anterior part of inter-atrial septum about half-way down its extent. At this point the septum fails to join the anterior wall of the atria which are here separated only by endocardium. × 25.

Fig. 4. Section through lower part of right atrium. The right and left venous valves have fused. × 25.

Fig. 5. High power view of right venous valve to show the cardiac muscle fibres in its substance. × 625.

Fig. 6. Transverse section through the upper part of the right atrium to show the position of the sinu-atrial node. × 17.

PLATE 2

Fig. 7. Transverse section through sinu-atrial node. The node, which forms almost the entire thickness of the right atrium, is traversed by the central artery. ×55.

Fig. 8. Sinu-atrial node. The fibres are clearly seen to be cross-striated. ×900.

Fig. 9. Section through atrio-ventricular junction. The atrio-ventricular bundle is here seen straddling the inter-ventricular septum. ×55.

Fig. 10. Transverse section through atria. The atrio-ventricular node is present in the inter-atrial septum in front of the opening of the coronary sinus, immediately behind a recess from the wall of the aorta. ×20.

Fig. 11. Section through the inter-atrial septum at a slightly higher level than that shown in Fig. 3. ×17.

Fig. 12. Subendocardial Purkinje fibres of the left limb of the atrio-ventricular bundle cut in transverse section. ×350.

PLATE 3

Fig. 13. Subendocardial Purkinje fibres of the right limb of the atrio-ventricular bundle. These fibres are cut in longitudinal section and show transverse striation. ×450.

Fig. 14. Purkinje fibres in the depths of the inter-ventricular septum. These fibres do not show transverse striation. ×500.

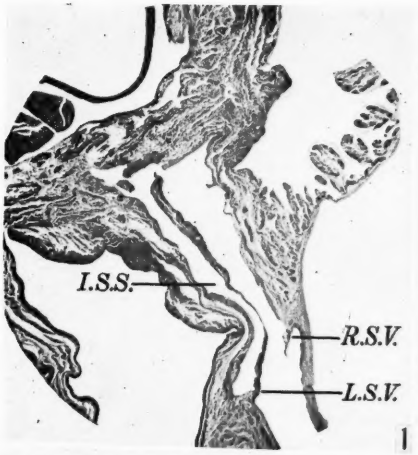
Fig. 15. Nerve endings in the sinu-atrial node. ×400.

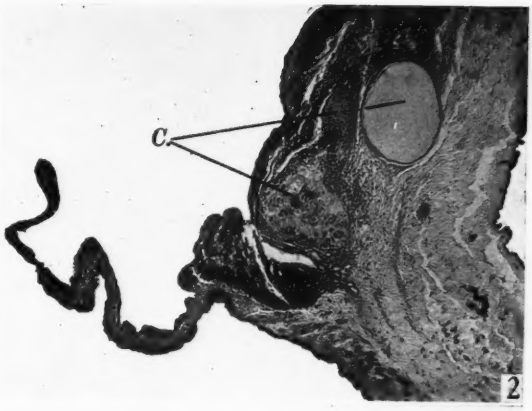
Fig. 16. Ganglion of inter-atrial septum. ×900.

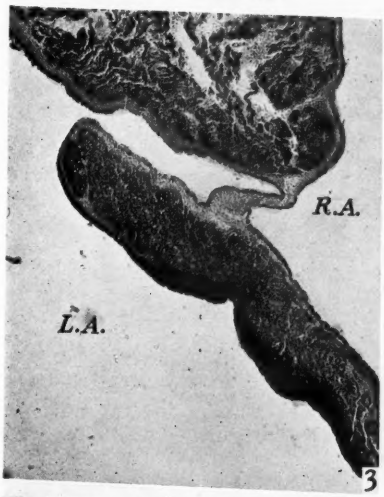
Fig. 17. Ganglion of inter-atrial septum. In top right quadrant a clump of the small, round, dark cells can be seen. ×500.

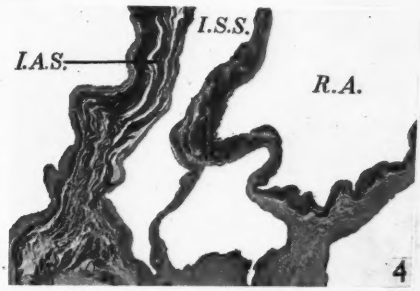
ABBREVIATIONS

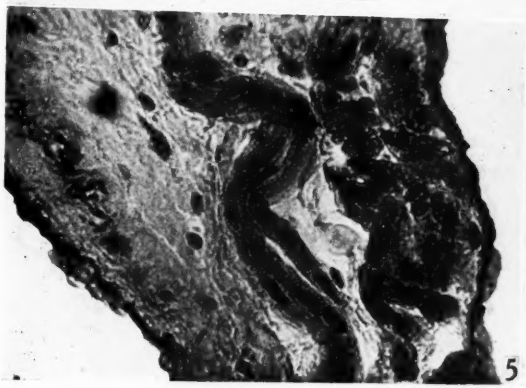
A.R. aortic recess.
A.V.B. atrio-ventricular bundle.
A.V.N. atrio-ventricular node.
C. cartilage.
C.S. coronary sinus.
E. endocardium.
I.A.S. inter-atrial septum.
I.S.S. intersepto-valvular space.
I.V.S. inter-ventricular septum.
L.A. left atrium.
L.L. left limb of atrio-ventricular bundle.
L.S.V. left sinus valve.
N.A. nodal artery.
R.A. right atrium.
R.L. right limb of atrio-ventricular bundle.
R.S.V. right sinus valve.
S.A.N. sinu-atrial node.
S.T. sulcus terminalis.











WALLS—Conducting tissue in *Erinaceus*

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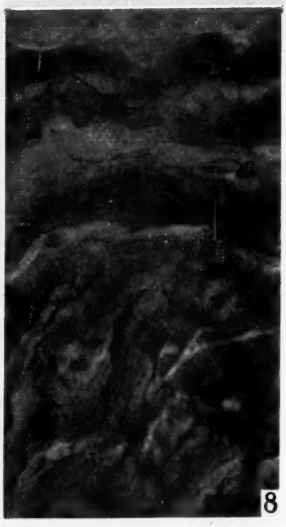
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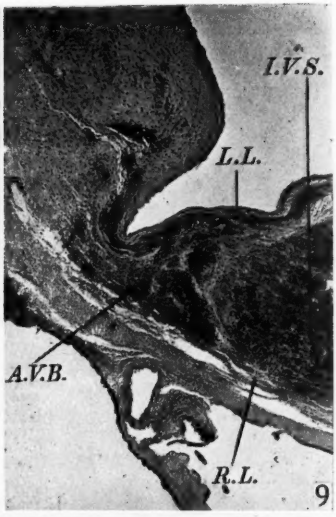
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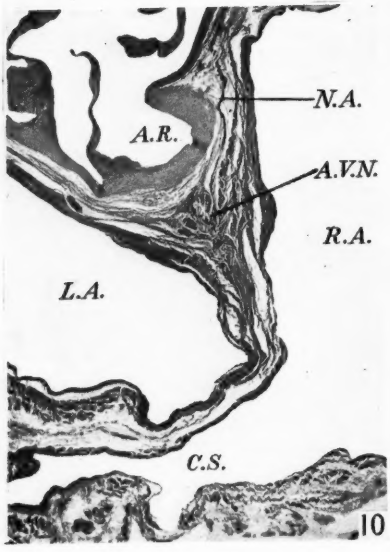
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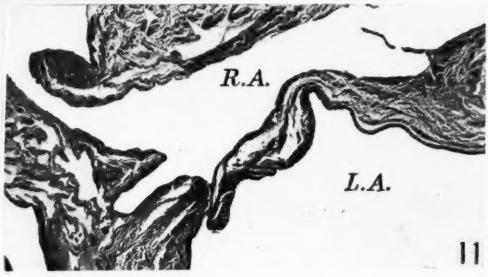
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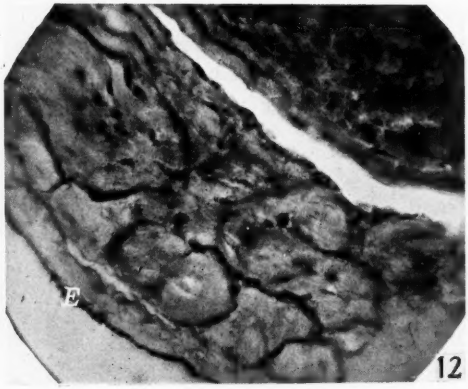
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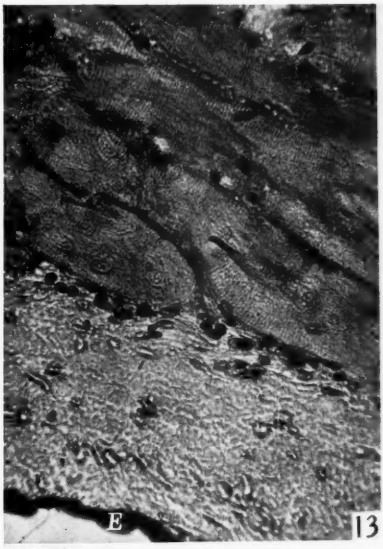

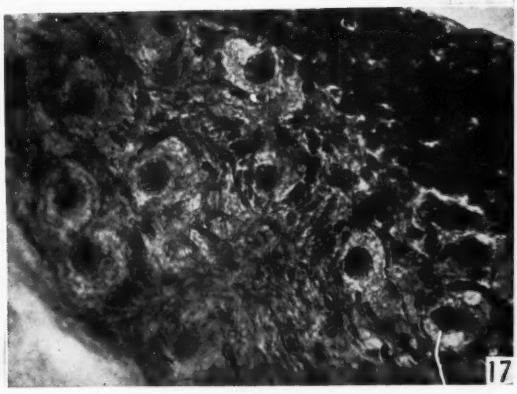

UNIV. OF MICH.

THE FATE OF SKIN HOMOGRAFTS IN MAN

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INTRODUCTION

Skin grafting has been practised by European surgeons for little less than a hundred years, and it is now the recognized method of treating extensive superficial injuries for which the autonomous process of repair would be too hazardous and too slow.

When, as sometimes happens, a patient has lost more skin than it is feasible to replace by the usual methods of autoplastic free grafting, the surgeon may choose between two alternatives. He may adopt an expedient such as autoplastic 'pinch grafting' (Davis, 1914) for making one piece of skin do the work of two or three, or he may decide to use skin from a relative of the patient or from a voluntary donor. The disadvantage of the first method is that the new skin is poor in quality and often unstable; and of the second, that its results are never permanent. It is true that, with few exceptions, the early investigators made no critical distinction between the fate of native and foreign skin (see Ehrenfried, 1908; Davis, 1910), and that skin homografts are still occasionally used by surgeons to-day without a clear recognition of their ultimate fate. Nevertheless, it has come to be acknowledged† that foreign skin cannot be used to form a permanent graft in human beings, except, without doubt, between monozygotic twins (Bauer, 1927; Padgett, 1932; Brown, 1937).

Theories about the mechanism that underlies resistance to homoplastic grafting are of two general types. The first is that the reaction is primarily *local* and *cellular*, and that evidence for the failure of homografts is to be found within the grafts themselves or in their immediate neighbourhood. This view is based mainly on histological considerations, and is held, together with a more detailed theory about the special function of the lymphocyte, by Loeb and his colleagues (Loeb, 1930, 1937). The second, long familiar to students of tumour transplantation (see Woglom, 1929), is that resistance to homoplastic grafting is *systemic* and primarily *humoral* in nature, and that in one form or another it follows the general pattern of an antigen-antibody reaction (Schoene, 1912*a*; Underwood, 1914; Holman, 1924; Todd, 1930; Landsteiner, 1931; Stone, 1942; Brown & McDowell, 1942). This view is in accord with general serological theory (see Landsteiner, 1936), and is based on the idea that the proteins and possibly other ingredients of the grafted tissues are sufficiently unlike those of the recipient to act as iso-antigens.

The observations recorded in this paper favour this second hypothesis; but our chief object has been to try to construct a continuous record of the behaviour of pinch homografts in man from a controlled study of their fate in a single patient. 'Pinch grafts' are discs 3 or 4 mm. in diameter prepared by raising the skin with sharp towel forceps or a fine needle, and slicing off the hillock so raised by a horizontal incision through its base. After a sufficient number have been cut, they are evenly spaced two or three diameters apart over the surface of the area to be grafted. Biopsy specimens can be removed at each change of dressings with only minor inconvenience to the patient; there is the very least danger of confusing homograft with native epithelium, or the grafts themselves with their controls; and the fact that the grafts are small and bedded in richly vascular tissue accentuates and probably accelerates the retrogressive changes undergone by foreign skin. For these reasons, pinch grafts are more suitable than continuous sheet ('split skin') grafts for observations of the type recorded here.

DESCRIPTION OF THE CASE

The patient (M. H.), an unmarried woman of 22 on whom the observations to be described below were made, was admitted to the Glasgow Royal Infirmary 1½ hr. after sustaining extensive deep thermal burns involving the right side of the chest posteriorly, the right flank, and the posterior aspect of the right upper arm. Round the more deeply burnt areas, simple blister burns extended medially as far as the left side of the back of the chest. The central parts of the burn were deeply coagulated and in some areas superficially charred. The burns were thoroughly cleaned with a 1·5% cetyl trimethyl ammonium bromide solution (Barnes, 1942) and covered with the first sulphanilamide-cod-liver oil emulsion dressing of a series applied every subsequent third or fourth day until the first grafting operation.

During the month after admission, the patient's condition was extremely poor; but some improvement followed the transfusion of a pint of blood on

* Working under a grant from the Medical Research Council.

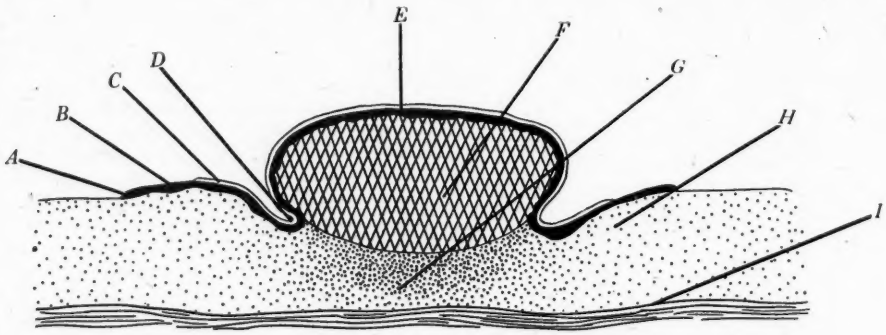
† See Lexer (1911), Gatch (1911), Schoene (1912*a*, *b*), Perthes (1917), Lexer (1919), Holman (1924), Blair & Brown (1929), May (1932), Mauclaire (1932), Padgett (1932), Trusler & Cogswell (1935), etc. The work of some of these authors is referred to in reviews by Fomon (1939), Willis (1939), and Binhold (1939). Willis and Binhold are not satisfied that the possibility of using homografts has been refuted.

the 29th day. By the 41st day the last of the sloughs had separated, and the burnt area was covered with raw, deep, richly vascular granulations (Pl. 1, fig. 1). Had the burns been smaller in area and the patient's condition better, it would at this stage have been possible and highly desirable to cover the raw areas with autoplastic split-skin grafts. Since a very marked improvement in the health and morale of burn patients usually follows the epithelialization of raw areas, the compromise decided upon was to cover part of the granulations with autoplastic pinch grafts, and another part with pinch homografts from the patient's brother (R. H.). The homografts were expected to overgrow the granulations and to form a suitable dressing for as long as the foreign epithelium survived. Accordingly, on the 45th day, fifty-two pinch autografts were removed from the patient's thigh and transplanted to the right lower and posterior side of the chest, and fifty pinch homografts from her brother's thigh were applied over and immediately below the right scapular region (see Pl. 1, fig. 2). One specimen from each set was reserved for histological examination by the routine described below. The pinches of both sets were symmetrically spaced about 1 cm. apart over a light dusting of sulphanilamide powder on the surface of the granulations, and were then held in place by a tulle-gras and elastoplast pressure dressing.

There still remained an extensive raw area for which we hoped to provide a temporary epithelial covering. Accordingly, on the 15th day after the first operation, a second set of twenty-eight pinch homografts was removed from the same donor and applied in the manner already described to a raw area above and to the left of the one on which the autografts had been planted. By the 36th day the coalescence and outgrowth of the autograft pinches, the reduction of the raw area by ingrowth from its sides, and the general improvement in the patient's health made definitive grafting possible. Neither set of homografts had grown sufficiently well to justify the hope that they would form an efficient temporary dressing. They were therefore scraped off with the granulations immediately before the raw surfaces were covered with thin dermatome-cut Thiersch grafts by Mr A. M. Clark. The patient was discharged 36 days after this final operation.

In this paper we are only concerned with the behaviour of the autografts and the first and second set of homografts in the 36 days between the initial operation and the final grafting. At each change of dressings during this period the surface was swabbed for bacteriological tests, and the grafts examined carefully by naked eye and photographed in colour and black-and-white. A single biopsy specimen, chosen so as to be as far as possible representative of its class, was removed under procaine anaesthesia from the autograft set, the first set of homografts, and (in the latter part of the period) from the second set of homografts as well. An autograft control is lacking only for the 36th day, when the removal of a biopsy specimen was felt to be no longer justifiable; but it proved possible to obtain six specimens from the first set of homografts at the 36th day, and two specimens from the second set.

The biopsy specimens taken at each dressing were fixed within half an hour of removal in Zenker's fluid without acetic acid, and treated in parallel through clearing in cedar-wood oil to imbedding in hard paraffin wax. Each



Text-fig. 1. A 'pinch graft' as it appears in median vertical section shortly after outgrowth of epidermal epithelium has begun. *A*, the margin of new growth; *B*, the *outer ring* of the newly spread epidermis, without a cuticle; *C*, the *inner ring* of the newly spread epidermis, with a developed cuticle; *D*, the 'corner' of the graft; *E*, the epidermis over the graft roof, where it is securely united to the underlying dermis; *F*, the collagen bundles of the dermis; *G*, the granulation tissue immediately underlying the dermis of the graft, i.e. the 'graft bed'; *H*, granulation tissue; *I*, a fascial layer (never included in the biopsy specimens).

specimen was cut serially at 10μ, and a number of evenly spaced though disconnected series were prepared by mounting every successive 25th section in a fixed order on the slides. A series of sections from each biopsy specimen was then stained with haematoxylin and eosin; with Masson's haematoxylin-Ponceau-light green; and with van Gieson's picro-fuchsin stains. In a subsidiary series of slides, median sections from each of the biopsy specimens removed at any one dressing were mounted on a single slide and stained optimally for the autograft representative, in order to see if the autografts and homografts differed in their general staining reactions. At no time, however, did this prove to be the case.

Pinch grafts are cut in such a way that their thickness increases from that of the epidermis at the edge to that of the full depth of skin in the middle. In spite of mounting on stout cardboard, the effect of fixation is therefore to make the pinches contract symmetrically but unequally and become button-shaped. This button-like shape is at first sight confusing: the edges of the graft bite so deeply into the soft granulation tissue that an epithelial layer may be cut twice in vertical section. Text-fig. 1 illustrates the general anatomy of a pinch graft as it appears in median vertical section shortly after outgrowth from its sides has begun, and it defines the topographical terms used in the detailed descriptions which follow. All our graft specimens represent developments of or deviations from this basic pattern.

DETAILED HISTORY OF THE CASE

Application of the grafts. The number, disposition, and method of application of the autografts and the first set of homografts have been described above. Specimens from each were examined histologically to ensure that they were of normal skin. The distribution of the raw areas before the grafts were applied is illustrated by Pl. 1, fig. 1.

Three days after grafting the elastoplast pressure dressing was removed. Inspection of the graft site through the meshes of the tulle showed that there had been very little exudation from the raw surface. Flora: *B. proteus.*

Fifth day. All the grafts had taken, and all were well vascularized (Pl. 1, fig. 2). There was no sign of epithelial outgrowth, and it proved impossible to draw any distinction between the two sets of grafts by naked-eye inspection. A biopsy specimen from each set was removed. Flora: *B. proteus.*

Autograft (A-5). A thin sheet of epidermal epithelium with somewhat flattened and elongated cells had spread over the granulation tissue to a width equal to about half the diameter of the graft on either side. In the outer ring of this new growth, only the basal layer cells immediately overlying the granulation tissue were cytologically normal. The staining reactions of the cells in the intermediate layers were feeble and imprecise and many of their nuclei were pyknotic. The cells in the surface layer were clearly keratinized, but retained their nuclei; no mature stratum granulosum or cuticle had been formed. Polymorph nuclei were identifiable in the epithelial layers.

(New epithelium overgrowing granulation tissue is invisible to the naked eye until it has developed a cuticle. Here and below, this fact accounts for certain obvious inconsistencies between the naked-eye and histological reports.)

The new growth was not symmetrical: at one point the cuticle over the roof of the graft dipped down into direct contact with naked granulation tissue. At this point (Pl. 2, fig. 7) there was a collection of multinucleate giant cells, some of which had wandered on to the surface of the graft. Isolated multinucleate cells were found elsewhere under the newly formed epithelium.

The epithelium over the graft roof itself was cytologically normal, but thinned out in two patches to a layer only two or three cells thick. Over one patch a small blister filled with clear exudate had formed between the granular layer and the stratum germinativum; over the other, a blister had formed within the Malpighian layer itself. The latter was roofed by a scaffolding of histologically intact but dead epithelial cells with empty nuclei, overlying an exudate containing a few pus cells, lymphocytes, and mononuclears.

The dermis of the graft was well vascularized, and the blood vessels stood out clearly in the collagen bundles. The collagen bundles themselves were sharply demarcated from the granulation tissue of the graft bed, and had not been infiltrated by any significant number of extraneous cells either from the blood vessels in the graft or from the underlying granulation tissue. The mesenchyme-cell population of the granulation tissue as a whole was sparse, and was made up of lymphocytes, plasma cells, polymorphs and mononuclears in that order of frequency. The population was fairly homogeneous and not appreciably denser in the immediate neighbourhood of the graft.

Homograft (H-5). In general histological appearance the homograft was closely similar to the autograft. Outward migration of epithelium was confined, however, to the immediate neighbourhood of its rim. The cells of the newly spread epithelium had the same cytological appearance as those in a corresponding position in the autograft; but infiltration by polymorphs, though nowhere intense, was more strongly marked. The epidermis over the graft roof was normal but for the presence of two blister patches precisely comparable with those described in the autograft; and the multinucleate giant cell reaction round the rim of the graft was equally well pronounced.

The dermis of the graft was well vascularized, but there had been a strongly marked infiltration of the collagen bundles by lymphocytes and mononuclears from the vessels in the graft and from the graft bed. This gave the dermis an 'eaten into' appearance; but in fact the collagen bundles were normal in packing and had lost none of their specific staining power.

The cell population of the graft bed, though not of the granulation tissue on either side, was denser than in the autograft, and plasma cells and polymorphs were relatively more abundant. There was a single patch of phagocytes with irregular foamy cytoplasm and one to four nuclei in the granulation tissue; but it bore no obvious anatomical

relationship to the graft, from which it was separated by a tract of 'typical' granulation tissue.

Tenth day. **It proved impossible to make any precise naked-eye distinction between autografts and homografts. The autograft pinches had rather less distinct margins, suggesting that some outgrowth over the granulations had taken place (Pl. 1, fig. 3). Flora: *B. proteus*.**

Autograft (A-10). New epithelium, thicker than that described under A-5 but similar in cytological appearance, had spread symmetrically over the granulation tissue to a distance equal to about two-thirds of the diameter of the graft. True cuticle formation had begun in the inner ring of this new growth, but the outer ring lacked a mature stratum granulosum and cuticle. The staining power of nuclei and cytoplasm became progressively feebler towards the margin of the new growth; occasional nuclei were pyknotic; and some polymorph infiltration had taken place (Pl. 2, fig. 8). The epithelium over the graft roof was normal in character, but the graft had been removed and fixed in the course of a cycle of desquamation. In vertical section it was therefore possible to distinguish Malpighian layer, stratum granulosum, cuticle, dead Malpighian layer, and old cuticle in that order from the inside outwards. The space between old epidermis and new was probably exaggerated by histological treatment, but was in any case free from pus and extraneous cells.

A multinucleate giant-cell reaction was found to be in progress wherever cuticle came into contact with naked granulations; and occasional small multinucleate cells were scattered under the new epithelium.

The dermis was cytologically normal, well vascularized, and free from extraneous cells. The cell population in the granulation tissue surrounding and underlying the graft was sparse, and was made up chiefly of lymphocytes and polymorphs in approximately equal numbers. Plasma cells were abundant in the graft bed, where an orientated fibroblast reaction was in progress, and new collagen had been laid down (compare Pl. 2, figs. 10, 15).

Homograft (H-10). Epithelium from the graft had spread less far over the granulations than in the autograft, and in a thinner sheet. Its general cytological appearance was, however, the same (Pl. 2, fig. 9). The viable epithelium over the graft roof was cytologically normal, but the restratification of the epidermis described in A-10 was here more pronounced; the space between old epidermis and new was congested with pus, and a small number of pus cells had prised their way into the superficial layers of the new cuticle (Pl. 2, fig. 11).

Multinucleate giant cells were found in the granulation tissue in relation to patches of cuticle.

Compared with H-5, perivascular infiltration of lymphocytes and mononuclears was very much less pronounced; and invasion of the dermis through its inner surface was confined to a few plasma cells. The 'eaten into' appearance was therefore lacking. An orientated fibroblast reaction was in progress in the graft bed (Pl. 2, fig. 10) and new mature-staining collagen had been laid down.

The general mesenchyme-cell reaction in the granulation tissue was more pronounced than in the autograft, and was complicated by the presence of fat spaces which in many cases were lined with large, spherical, and sometimes multinucleate fat phagocytes. The cell reaction in the graft bed was likewise more intense than in the autograft, and plasma cells were relatively more abundant. The blood vessels in the granulation tissue were congested with polymorphs and eosinophils.

Fifteenth day. **The homografts appeared to be healthy, and there had been some visible outgrowth from those nearest to the edge of the raw area, where several had coalesced. Only a few of the grafts in the centre of the raw area showed signs outgrowth visible to the naked eye. Almost all the autografts had grown, and in the centre of their area many neighbouring grafts had coalesced. Flora: *B. proteus*.**

After removal of the biopsy specimens, twenty-eight further pinch homografts were removed from the thigh of the original donor (R. H.) and transplanted to the centre of the remaining raw area on the back over a light dusting of sulphanilamide powder. Tulle-gras and saline dressings were applied: a pressure dressing was not technically feasible. One specimen was reserved for histological examination and found to be normal in character.

Autograft (A-15). There had been widespread symmetrical outgrowth of new epithelium well beyond the margin visible by naked-eye inspection of the biopsy specimen. The epithelium in the outer ring of new growth, where no cuticle had yet formed, had the combined degenerate and immature appearance already described in detail in A-5 and A-10. Polymorph infiltration was most strongly marked where the epithelium was thickest (Pl. 2, fig. 12). The cuticle over the graft was thick and in some areas enclosed small pockets of pus.

Very few small multinucleate phagocytes were identified.

The cell population of the granulation tissue as a whole (plasma cells, lymphocytes, and polymorphs, with a few fat-droplet cells) was sparse; but in one area, bearing no obvious anatomical relationship to the graft, there was an extremely dense aggregation of polymorphs. An orientated fibroblast reaction in the graft bed and immediately under the newly spread epithelium had been accompanied by the laying down of new mature-staining collagen fibres.

Homograft (H-15). The homograft specimen showed clear evidence of retrogressive changes. On one side of the graft (as it appeared in median vertical section) epithelium which had formerly overgrown the granulations was represented only by a thin detached band of still coherent cells which had lost all staining power. On the other side, newly spread epithelium was still present; but it had disengaged from the underlying granulation tissue and folded over backwards on itself. The cells were swollen—an appearance doubtless exaggerated by part of the free-lying epithelium being cut obliquely—and had lost almost all their staining power; but it proved impossible to distinguish any cytological abnormalities in the freshly disengaged epithelium near the central pinch (Pl. 2, figs. 13, 14) which were not shown to an equal or only slightly lesser degree by the

marginal epithelium of the autografts. Neither in the free-lying epithelium itself, nor in the granulation tissue immediately underlying the newly spread epithelium was it possible to detect any significant *local* aggregation of mesenchyme cells; and the polymorph infiltration of the epithelial layers was no more strongly marked than in the autograft. The evidence for a retrogressive change consists in all but total loss of epithelium from one side of the specimen, and its disengagement from the underlying granulations on the other.

The epithelium over the graft roof was cytologically normal. The cells of the corneal layer were, however, more rounded than is usual, and most of them retained eleidin granules and traces of nuclear staining power.

Escape of lymphocytes and mononuclears from the blood vessels into the dermis of the graft was no more strongly marked than in H-10, but histiocytes had accumulated in the granulation tissue of the graft bed, and a few of these, together with plasma cells, had infiltrated the inner surface of the dermis. The packing of the collagen bundles was normal, and none had lost their specific staining power. The margin between the collagen bundles and the granulation tissue was therefore sharply defined; and in some areas the margin was made more precise by the presence of blood vessels flattened against the underside of the dermis.

As in H-10 the general mesenchyme cell reaction in the granulation tissue was complicated by the presence of fat spaces and islands of fat phagocytes separated from the underside of the graft by tracts of typical granulation tissue. The reaction as a whole was more intense than in the autograft, and the cell population was different: polymorphs were infrequent, plasma cells and lymphocytes relatively more abundant. A fine tracery of new mature-staining collagen fibres was easily identifiable among the plasma cells in the graft bed (Pl. 2, fig. 15).

Twenty-third day. **The autografts had now coalesced into a continuous sheet of feebly cuticularized epithelium studded with the outlines of the original pinches. The homografts of the first set showed clear signs of retrogression; each was dead white in colour and lifted off from the granulations; and, with the exception of two neighbouring pairs near the upper margin which had coalesced, none was surrounded by a macroscopically visible rim of newly spread epithelium. Some of the marginal grafts had been overgrown by the patient's own skin from the margin of the raw area (Pl. 1, fig. 4).**

Only twenty of the 2nd-set homografts, now 8 days old, were adherent, and of these four were represented only by a translucent filmy patch. In external appearance, the remainder differed from the homografts of the first set after 5 or 10 days only in being more opaque and somewhat lifted up from the granulations (Pl. 1, fig. 4). Flora: *B. proteus*.

A biopsy specimen was removed from each of the three sets of grafts, the specimen from the autograft set being cut from the edge of the sheet in order to include the margin of new growth.

Autograft (A-23). The greater part of the specimen was roofed by mature cuticularized and cytologically normal epithelium. Some blistering of the epidermis had taken place, and small pockets of pus were occasionally enclosed between the old epidermis and the new. The epithelium of the growing edge (Pl. 3, fig. 16) showed the combined features of immaturity and secondary pathological change described above in detail. The epidermis of the inner ring of new growth was normal in all but the thickest regions, where nuclei were occasionally pyknotic and polymorph infiltration had occurred (Pl. 3, fig. 17).

Epithelium of sweat and sebaceous glands was identifiable in the dermis of the graft. Collagenization of the graft bed and the surrounding granulation tissue was more strongly marked on one side of the graft than on the other, but was in any case further advanced than in A-15. The cell population of the granulation tissue was moderately dense, and was made up chiefly of plasma cells and polymorphs.

Homograft (*1st set*) (H-23). Epithelium which had formerly overgrown the granulation tissue was now represented only by isolated strands of cuticle invested by multinucleate giant cells. Over the graft roof the epidermis had separated from the dermis and had undergone gross pathological changes which decreased in severity from the inside outwards. The cells of the basal layers had separated from one another and from the underlying collagen and had undergone complete nuclear lysis. The cells of the more superficial layers retained their anatomical connexions, but their nuclei had disappeared or had undergone sharp pyknosis. A few wandering cells had invaded the dermo-epidermal cavity, though they were present there in no greater numbers than in the collagen bundles of the underlying dermis (Pl. 3, figs. 18, 19).

Exactly similar changes, including the separation of the basal layer cells from one another and from the underlying dermis, had occurred in the deep lying and therefore protected cells of a hair follicle belonging to the original graft.

At the corners of the graft cuticle cells were in direct contact with granulation tissue, and direct evidence of phagocytosis by multinucleate giant cells was found.

There were patches of pus over the exposed part of the dermis, where areas of the epidermis had broken away, and polymorphs from the outside working their way inwards had infiltrated the superficial bundles of the collagen. The dermis as a whole had the lifted-up appearance observed by naked-eye inspection, but was substantially normal in bundle packing and staining power. There had been a feeble fibroblast reaction in the graft bed, though not in the granulation tissue on either side, and no new mature-staining collagen fibres were found. Histiocytes had accumulated in small numbers round the lower surface of the dermis, but there had been little invasion of the collagen bundles. The cellular reaction in the granulation tissue was feeble, though the blood vessels had thickened walls and were congested with polymorphs. Immediately under the graft the cell population was denser than in the autograft, and as before plasma-cells were the dominant cell type.

Homograft (*2nd set*) (H2–8). Inspection of the biopsy specimen immediately before fixation (made as a matter of routine to secure proper orientation in the block, etc.) suggested that the epidermis formed a somewhat loose-

fitting lid to the graft; but in other respects, the specimen did not differ in any clear-cut way from the 1st-set homografts after 5 (H-5) or 10 (H-10) days.

Microscopical examination revealed a different state of affairs. The entire epidermis of the graft was lifted off from the underlying dermis and had undergone gross erosion of a type closely comparable to that described in H-23. The cells of the deeper layers were macerated, but in this case the dermo-epidermal cavity was congested with pus. The majority of the cells in the epithelial layers had empty nuclear spaces (Pl. 3, fig. 20), and in the remainder the nuclei were sharply pyknotic (Pl. 3, fig. 21). The extraneous cell reaction was confined to the space between dermis and epidermis; extensive tracts of the necrotic epithelium itself were free from foreign cells except for an occasional degenerating polymorph nucleus.

There was no evidence, as there was in H-23, that epithelium had at any previous time overgrown the granulations. Multinucleate giant cells were found only at the corners of the graft in relation to patches of still adherent cuticle.

The collagen of the dermis had been heavily infiltrated by polymorphs from the dermo-epidermal cavity; but although the bundles were prised apart their specific staining power remained normal (Pl. 3, fig. 22). Round the lower surface, however, many of the collagen fibres splayed out into finer fibrils with loss of specific staining power.

The granulation tissue surrounding the graft was grossly haemorrhagic, no doubt owing to the trauma involved in cutting this particular graft; but although the blood vessels were congested, the cell population (predominantly of polymorphs) was comparatively sparse. A feeble fibroblast reaction, without evidence of new collagen formation, was in progress in the graft bed, from which numerous polymorphs had invaded the inner surface of the dermis.

Thirty-first day. The autograft sheet, as it had now become, had not increased appreciably in area. A cycle of desquamation, seen at a brief routine inspection to have begun by the 26th day, was now complete.

The great majority of the 1st-set homografts showed no naked-eye evidence of the persistence of epithelium; each was represented by a small milky-white patch flush with or in some cases apparently imbedded in the granulation tissue. Only the two pairs of neighbouring grafts which had been seen on the 23rd day to have coalesced showed no outward signs of retrogression.

The homograft pinches of the second set were ragged and scrappy in outline, dead white in colour, and somewhat raised up from the granulations. Cuticularized epithelium, or at least cuticle, survived over many of them (Pl. 1, fig. 5). Flora: *B. proteus.*

No biopsy specimens were removed on this occasion.

Thirty-sixth day. Apart from a noticeable ingrowth of epithelium from the margin of the raw area, particularly in the neighbourhood of the 1st-set homografts, there was little change from the appearance described on the 31st day; but the two small coalesced patches of 1st-set homograft epithelium had increased in area. Many 1st-set homografts had been overgrown by the patient's own skin, and these had dark patches in the centre suggesting necrosis or haemorrhage (Pl. 1, fig. 6). Flora: *B. proteus.*

Immediately before the application of Thiersch grafts in the final grafting, the following biopsy specimens were removed:

H-36 A, B, C. Three separate specimens of the first set of homografts from the centre of the grafted area.

H-36 D. One of the two pairs of 1st-set homografts that had coalesced with each other.

H-36 E. A homograft of the first set from the margin of the raw area, overgrown by the patient's own skin epithelium.

H 2–17. Two adjacent 2nd-set homografts with the granulation tissue between them, imbedded and cut together.

The only foreign skin left in place at the final grafting operation was the second of the two pairs of coalesced 1st-set homografts. It later became incorporated in the patient's own skin, and was no longer recognizable as a separate island.

H-36 A. The fact that epithelium had formerly overgrown the granulations was betrayed by the presence of isolated adherent strips of cuticle, together with the tell-tale giant cells, far out over its surface (Pl. 4, fig. 24). The epidermis survived only in the form of a flattened pocket on one side of the graft (Pl. 4, fig. 25). The cavity enclosed between it and the dermis, from which it was widely separated, contained isolated red cells, polymorphs and mononuclears, together with fibrin and a certain amount of unidentifiable cellular debris. The epidermis itself retained no trace of nuclear staining power, though keratohyalin granules were still identifiable in the granular layer; and the greater part of it was wholly free from extraneous mesenchyme cells.

Extensive islands of large multinucleate phagocytes were found imbedded in the granulation tissue—in every case in association with strips of cuticle which the granulation tissue had overgrown.

The greater part of the collagen of the graft consisted of normally packed and normally staining fibres. There had, however, been an appreciable 'erosion' (see H 2–8) of the lower surface, with loss of specific staining power; and in this case the erosion had been accompanied by a feeble infiltration of histiocytes from the graft bed. Any new collagen that had been laid down in the graft bed was no longer recognizable as such. The general cellular reaction in the granulation tissue—predominantly of plasma cells—was feeble; nor did the population increase in density in the immediate neighbourhood of the graft. The vessels were however moderately congested, and each was surrounded by a halo of lymphocytes and mononuclears.

H-36 B. In this specimen, the dermis had been almost wholly overgrown by richly vascular granulation tissue, so

that the upper surface of the graft was exposed only by a circular aperture visible in median vertical section (Pl. 4, fig. 26). Traces of epithelial debris were identifiable in the aperture.

As a result of this process of overgrowth, the cuticle from the original edges of the graft was deeply buried in the granulation tissue and invested by multinucleate giant cells (Pl. 3, fig. 23).

The margin of contact between the dermis of the graft and the underlying granulation tissue was sharply defined, and the collagen bundles were cytologically normal in appearance.

The cell population of the granulation tissue was moderately dense but evenly distributed.

H-36C. In all essential respects this specimen was precisely similar to H-36B.

H-36D. The epidermis roofing this specimen was thick and had a fully developed normal stratum granulosum. The corneal layer was, however, poorly or precociously developed. The cells of which it was built up, though clearly keratinized, retained sharply staining nuclei.

The collagen bundles in the two pinches were normal in packing and staining power; but in one of them the dermis was cup-shaped and enclosed a richly vascular tract of granulation tissue, populated mainly by histiocytes, between it and the epidermis. Many of the histiocytes were binucleate. A dense orientated fibroblast reaction was in progress under each graft, and abundant new collagen had been laid down. Histiocytes had infiltrated the inner surface of the dermis in isolated patches. Blood vessels, little congested, stood out cleanly in the granulation tissue, which was populated by histiocytes, lymphocytes and plasma cells in approximately equal numbers.

H-36E. The epithelium bounding this specimen, of native origin, was cytologically normal and had a fully developed normal cuticle.

The dermis was cup-shaped, with the concavity facing outwards in such a way as to enclose a richly vascular and highly haemorrhagic tract of granulation tissue populated mainly by histiocytes, many of which were binucleate (compare H-36D). The ground substance was necrotic, and little fine detail could be worked out. Except in the immediate neighbourhood of the blood vessels, the cell population of the granulation tissue was exceedingly sparse. Unfortunately the graft bed itself was not included in the biopsy specimen.

H2–17. The two adjacent 2nd-set homografts included in this specimen did not differ from each other in any essential way, and the description which follows applies to both of them.

No trace of the epidermis could be found. The surviving dermis of the graft was bounded by a rather sharply defined superficial layer of collagen fibres which had lost their specific staining power, though a few mature-staining fibres could be found among them (Pl. 4, fig. 27). The necrotic layer was infiltrated with polymorphs. The dermis as a whole was highly eroded, and had been reduced to a thin cup with the concavity facing *inwards* over highly vascular granulation tissue. Fibre bundle packing was no longer compact. The cell population of the granulation tissue was chiefly made up of polymorphs and mononuclears; the blood vessels stood out cleanly and were uncongested.

REVIEW OF THE EVIDENCE, WITH A CONSIDERATION OF ONE FURTHER CASE

It is unwise to be dogmatic about the behaviour of skin grafts in general merely from the study of a single case, particularly in view of the fine gradations in the responses made by different human beings to the transplantation of foreign skin. Nevertheless, we hope that the analysis which follows will make a suitable base-line for future investigations, to be amplified or corrected as the case may be.

(*a*) *The inflammatory reaction.* The homografts rapidly provoke a mesenchyme cell and vascular reaction which has most of the features of a non-specific inflammatory process; e.g. the perivascular infiltration of H-5, likewise found in the reaction to heteroplastic 'grafts' of *purified* collagen (Pullinger & Pirie, 1942), and the quantitatively greater general cellular reaction in the graft bed and the surrounding granulations. Both subside to a large extent while the dermis of the grafts is still intact, and even before the epidermis has finally disappeared.

(*b*) *Dermis and collagen.* The collagen bundles of the dermis of homografts proved to be highly resistant. It is only in the later stages that erosion of the lower surface—a splaying-out of the collagen bundles with loss of specific staining power—becomes visible (H-36A, H2–17); and it may or may not be accompanied by a histiocyte or other mesenchyme-cell reaction in the immediate neighbourhood. No direct evidence of collagen phagocytosis was recorded in case H; perhaps because 36 days was an insufficient time for the reaction to develop in full.

In another case (F) we have studied, however, pinch homografts had been allowed to persist for 2 months before definitive grafting; that is to say, long after the foreign epithelium which at one time covered the whole of the raw area had disappeared, and when the foreign collagen was represented only by a small patch fully overgrown by and imbedded in soft granulation tissue (Pl. 4, fig. 28) in the manner foreshadowed by H-36 B and C. Two separate biopsy specimens removed at the final grafting showed the clearest direct evidence of collagen phagocytosis: the space formerly occupied by the bulk of the dermis was populated by mononuclear phagocytes, their cytoplasms loaded with one, two or three fragments of material that often (Pl. 4, fig. 31) retained traces of a fibrous structure and which responded sharply to Masson's phosphomolybdic acid-light green and van Gieson's picro-fuchsin stains (Pl. 4, figs. 29–32).

We may infer that a straightforward reaction of phagocytosis is in part responsible for the final and complete removal of the foreign collagen. Probably it is not the only mechanism involved: case F exhibits a process of erosion so strongly marked that it can only be described as one of fading away. Mesenchyme cells seem to have played no part in the reaction: the collagen bundles splay out, lose

their staining power, and merge imperceptibly into the reticular ground substance of the granulation tissue (Pl. 4, fig. 33).

Autograft collagen, on the other hand, persists. A brisk orientated fibroblast reaction occurs in the neighbourhood of the graft, and this is associated with the laying down of similarly orientated tracts of new mature-staining collagen in the graft bed, and, later, under the newly spread epithelium. A similar reaction begins in the homografts (H-10, H-15; Pl. 2, figs. 10, 15) but later subsides, so that the graft bed returns to its original state of soft granulation tissue. This overgrows and imbeds the remaining collagen: a process almost complete in H-36 B and C (Pl. 4, fig. 26) and complete in case F (Pl. 4, fig. 28) at the time of examination.

(*c*) *The foreign body reaction.* The cuticle cells of both autograft and homograft provoke a vigorous multinucleate giant-cell reaction where and wherever they come into contact with naked granulation tissue. It subsides in the autograft, because the granulations are rapidly protected by epithelium of new formation. It persists longer in the homografts because the granulations are re-exposed by the dissolution of the epidermis, and because patches of cuticle from the margins of the graft tend to get overgrown by and imbedded in the granulation tissue (Pl. 3, fig. 23). Although it is often deceptively prominent, there is no reason to describe the process otherwise than as a non-specific foreign body reaction (Pl. 2, fig. 7; Pl. 3, fig. 23; Pl. 4, fig. 24).

(*d*) *Desquamation and blister formation.* Both autografts (A-5, A-10) and homografts (H-5, H-10) undergo cycles of desquamation (or restratification) about a plane of cleavage in the Malpighian layer (Pl. 2, fig. 11). The space between the old and the new epidermis may or may not become filled with pus. The blisters described in some detail in A-5 and H-5 may represent the beginnings of such a cycle, since it was clearly complete at 10 days. On the other hand, their presence may be connected with the trauma involved in the original cutting of the graft.

(*e*) *The epidermis.* The dissolution of foreign epithelium, unlike that of the collagen, is sudden in onset and rapid in execution. The difference between homograft and autograft epithelium after 5 and 10 days, though in both cases in slight favour of the autograft, lay well within the normal range of variation of either. After 15 days, however, loss of epithelium was far advanced in the zone of new growth (H-15); and what peripheral epithelium remained was degenerate in appearance and had lost its anatomical contact with the underlying granulations (Pl. 2, figs. 13, 14). By 23 days, degeneration of the epithelium has spread to and is virtually complete in the graft roof itself (Pl. 3, figs. 18, 19); and the 36-day specimens show inconstant traces of necrotic cell-remains alone (Pl. 4, figs. 25, 26).

So far as can be judged from the 23- and 36-day specimens (and from H2–8), the process begins in the basal layers of the epidermis and spreads superficially. It could hardly do otherwise, whatever mechanism was involved. But for exactly this reason, no special significance can be attached to the detailed cytological changes visible in the homograft epithelium even quite early (H-15) in the process of breaking down (Pl. 2, figs. 13, 14). If the basal layer cells are attacked first and break up or disengage, the pathological changes undergone by the cells in the more superficial layers will be largely due to autolysis consequent upon the loss of blood supply and oxygen. They will, in any event, be secondary. Nor would it be easy to identify primary cytological changes of a subtle character in the epithelium newly spread over granulation tissue (as in H-15). Epithelium of new formation is wholly atypical both in cytological and histological anatomy, as the illustrations of the A-series (Pl. 2, figs. 8, 12; Pl. 3, figs. 16, 17) were designed to show;* and its outer ring, which has not developed a true corneal layer, is unprotected from the pulling and rubbing of the dressings as well as from the doubtless unfavourable action of the exudates that accumulate beneath them.

What is wanted, then, is evidence of a primary reaction directed against the basal layer cells of the epidermis over the graft roof—where it is securely united to the underlying dermis by elastic and fine argyrophil fibres (Pautrier & Woringer, 1930; Szodoray, 1931). This evidence is lacking. The reaction had not yet begun in the 15-day specimen, and was far too advanced in the 23rd. We can therefore merely put on record the secondary consequences of its having occurred some not very long time beforehand. The one point that is quite clear is that no mesenchyme-cell reaction (e.g. of lymphocytes) played any necessary and significant part in the secondary changes we observed (Pl. 3, figs. 18, 19); and that there was no evidence, as there should have been, that it played any part in the hypothetical primary reaction that preceded them. A *humoral* mechanism, apparently, is responsible for the breakdown of foreign skin epithelium.

(*f*) *The behaviour of the 2nd-set homografts.* Naked-eye observation, reinforced by the evidence from the biopsy specimens (H2–8, H2–17), showed that the second set of homografts did not undergo the same cycle of growth and regression as the first. Primary adhesion was less secure (though this may have been due to the absence of a pressure dressing); growth of new epithelium was absent or excessively feeble; and degenerative changes were greatly accelerated in progress and in time of onset. Homo-

* The structure and behaviour of epithelia spreading over surfaces or creeping under scabs is known from the classical research of Loeb (1898) and Marchand (1901).

grafts of the first set were not markedly abnormal after 10 days, and specimen H-15 retained normal epithelium over the graft roof for at least 15; but the 8-day biopsy specimen from the second set (H2–8) is hardly distinguishable from its 23-day contemporary among the first. Likewise the two grafts of specimen H2–17 did not differ substantially from H-36 A, B and C. In short, the degenerative changes that affected the first set of homografts appeared to occur *simultaneously* in the second set; and not, as might have been expected, after an equivalent length of time.* The biopsy specimens themselves suggest that if a distinction is to be made between them, the 2nd-set homografts were even worse than their 1st-set contemporaries. If this distinction proved to be of general occurrence, it would not be surprising. Degeneration of the first set begins *after* new epithelium has grown, and *after* reparative changes—e.g. the laying down of collagen in the graft bed—have advanced to a certain degree. The 2nd-set homografts, on the contrary, start degenerating from scratch.

In contrast to the histological evidence, naked-eye inspection tended to give the benefit of whatever distinction could be drawn between 1st- and 2nd-set homografts to the latter. Specimen H2–8 (see p. 303) suggests a likely explanation for this: so long as cuticle persists, even as a 'loose-fitting lid' to the graft, it will naturally conceal the degenerative changes that are going on underneath it.

(*g*) *The revived homografts.* The careful naked-eye record of their origin and progress, and the symmetry of growth around them, suggest that the epithelium roofing and uniting the two coalesced grafts of specimen H-36D was of genuinely foreign origin. If this is so, this specimen (and the other which was left in situ at the final grafting operation) at least temporarily survived the degenerative changes undergone by all the others. The advanced autograft-like collagenization of the graft bed also speaks in favour of this view. On the other hand, the coalescence took place near the margin of the raw area, where native epithelium was rapidly growing in; and since its margin would not be visible, we cannot exclude the possibility that the epithelium was of native origin (as it clearly was in H-36E).

DISCUSSION

The breakdown of foreign epithelium as an immunity reaction. The absence of a demonstrable cellular mechanism, the time relations of the process as a whole, and the accelerated regression of the 2nd-set homografts combine to suggest that the breakdown of foreign skin epithelium is brought about by a mechanism conforming to the general pattern of an antigen-antibody reaction.

What may be called the 'immunity hypothesis' was transferred by Schoene (1912*a*) to the grafting reactions of normal tissues from what was then current theory in the subject of tumour transplantation. It has now been finally and conclusively proved that resistance to transplanted tumours provoked by the injection of foreign but homologous normal or malignant tissue 'breis' is directed against the foreign cells *as such*—irrespective of their malignant character (Bittner, 1936; Gorer, 1938; Woglom & Eisen, 1941); and some attempt has been made (Phelps, 1937; Gorer, 1938) to demonstrate the iso-antibodies concerned. In spite of the suggestive evidence of Underwood (1914) and Holman (1924), the immunity theory has fallen into abeyance among students of normal skin grafting, though there are signs (Stone, 1942; Brown & McDowell, 1942) that it is now being revived.

Disregarding the negative histological evidence, of which nothing more can be said, it is clear that the behaviour of 2nd-set homografts is all-important. Loeb (1930) pointed out that if immune bodies played any significant part in resistance to homoplastic grafting, then a second graft transplanted when the reaction to a first was at its height should be attacked with equal intensity at once. He had found no evidence that this was the case, and quoted the work of Fleischer (1918) in support of this negative conclusion. Fleischer, however, stated that the leucocytic reaction was 'very much more marked' when pieces of guinea-pig kidney, freed from cortex, were transplanted into subcutaneous pockets in other guinea pigs 10–12 days after the last of a series of injections of homologous liver cell suspensions. Results of a similar type have been reported by Schoene (1912*b*), Tschernischoff (1912), and Sokolof (1925), and have been interpreted in favour of an immunity hypothesis. Phelps (1937) found that the serum of rats immunized by homologous spleen cell injections contained what was presumably an iso-antibody causing lysis of spleen cell cultures from other rats; and Blumenthal (1939, 1941) reports that the systemic lymphocyte reaction in rodents and fowls is accelerated (though not apparently intensified) when a second homograft is transplanted after a first from the same source. None of these experiments is conclusive; but they are difficult to interpret otherwise than on the grounds of an immunity theory.

The prompt local inflammatory reaction (see p. 305) is not necessarily an obstacle to the immunity theory. One possibility is that it has the character of a local anaphylaxis. A reaction of this sort is sometimes produced when a dose of antigen is administered locally after immunity (or sensi-

* One of us (T. G.) had recorded a similar anomalous behaviour on the part of 2nd-set homografts in two earlier cases, and had framed the hypothesis which follows in the Discussion section to account for it.

tivity) has developed. In this case the antigen-producer (the graft) is present all the while immunity is developing; and the inflammatory response may be a reaction to immune bodies formed in small but increasing quantities shortly after the foreign skin has been grafted. It is true that the Arthus phenomenon occurs in a clear-cut form only in rabbits; but if there is any tendency at all for it to occur in other animals, no situation could be more ideal than that offered by the skin homograft.

In the case of a comparatively inert tissue like collagen, the cellular reaction (of phagocytosis) may be the final link in a chain of reactions of the immunity type. In the case of skin *epithelium*, we can only suppose that all the significant part of the process is purely humoral.

The significance of blood groups. The blood groups of donor (O) and recipient (O) in case M.H. were compatible; yet the retrogression of the transplanted epidermis could hardly have been more rapid and more complete. The belief that blood compatibility and tissue compatibility overlap or go hand-in-hand is the best known form of the immunity hypothesis (Davis, 1917; Masson, 1918); and many who have claimed successful results from skin homografting (e.g. Shawan, 1919; Baldwin, 1920; Dyke, 1922) have attributed their success to judicious grouping. The blood-group theory is favoured by expert serologists (Todd, 1930; Landsteiner, 1931; Wiener, 1939) because there exists a high degree of individual differentiation in mammalian blood, and because the group-specific substances have been identified in most of the tissues of the body (see Wiener, 1939). Unfortunately, they are said to be lacking from skin epithelium itself (Yosida, 1928). Most writers who support the blood-group theory do not make a clear distinction between two possibilities: whether the antigen complexes governing blood compatibility and tissue compatibility are the same, or whether they are merely of the same sort. Common to both, however, is the assumption that resistance to homografting is natural and not acquired; i.e. that the iso-antibodies are ready-made, as they are in the form of agglutinins in human blood, and do not have to be manufactured. If this is so, it is difficult to see why 2nd-set homografts should behave much differently from their predecessors.

The blood-group theory is not supported by the evidence from experimental homografting in fowls and rodents (Ingebrigtsen, 1912; Kozelka, 1933; Haddow, 1934; but see Gorer, 1938), nor by the more extensive clinical observations (Holman, 1924; Padgett, 1932; Shinoi, 1932; Binhold, 1939; Brown, 1941). Brown found that skin homografts never persisted 'in spite of attention to grouping down into the *M* and *N* subgroups'. Binhold claimed that 51 out of 167 experimental skin homografts between human volunteers were successful, irrespective of *A–B–O* and *M–N* group relationships. His grafts were examined between the 3rd and the 7th month after transplantation. Unfortunately, each subject received only three 'pinches' of foreign skin, and each pinch was separated from its neighbours and immediately bordered by normal skin. Without continuous histological checking, claims of success for homografts of this type must be received with the greatest caution. It is only too likely that the foreign collagen was overgrown by native epithelium, as in specimen H-36E. But even if we disregard Binhold's experiments, the balance of evidence shows that blood compatibility is not sufficient, and is not known to be necessary, to ensure compatibility of skin.

SUMMARY

1. A controlled investigation of the reaction made by a human patient to the transplantation of foreign skin is reported.

2. The patient had an extensive raw granulating area produced by a thermal burn. This was sown with fifty-two pinch autografts from her own thigh, and with fifty pinch homografts from her brother's. Biopsy specimens from both sets were removed at each change of dressings and examined histologically.

3. A second set of twenty-eight pinch homografts from the same donor was transplanted to a different part of the raw area 15 days after application of the first. Biopsy specimens from this set were thereafter examined in parallel with those from the other two.

4. The autografts grew in the fashion characteristic of pinch grafts, and coalesced to form a continuous sheet.

5. Growth from the first set of homografts was at first hardly distinguishable from that of the autografts; but degeneration of the epidermis was seen to have started by 15 days, and was virtually complete by 23.

6. The second set of homografts did not undergo the same cycle of growth and regression as the first: dissolution was far advanced by the 8th day after transplantation. The degenerative changes that affected the first set of homografts appeared simultaneously in their immediate contemporaries of the second set.

7. No evidence was found that the breakdown of foreign skin *epithelium* was brought about by or accompanied by a local reaction on the part of lymphocytes or other mesenchyme cells.

8. The foreign collagen was much more resistant than the foreign epithelium. Evidence from another case showed that it was eventually removed by a mechanism in which phagocytosis played a demonstrable part.

9. The time relations of the process, the absence of a local cellular reaction, and the accelerated re-

gression of the second set of homografts suggest that the destruction of the foreign epidermis was brought about by a mechanism of active immunization.

Special expenses involved in this work were defrayed by a grant to one of us (P. B. M.) from the Medical Research Council. We wish to record our deep gratitude to Dr Leonard Colebrook for his practical help, advice, and encouragement at all stages of this work; to Prof. J. W. S. Blacklock for his hospitality and his help in interpreting the histological material; and to Mr A. M. Clark for his friendly co-operation throughout.

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EXPLANATION OF PLATES

The letters in heavy type after the specimen numbers refer to the zones illustrated by Text-fig. 1.

Plate 1

Fig. 1. The distribution of the raw areas before grafting.

Fig. 2. Distribution and appearance of the autografts (*A*) and the homografts (*H*) 5 days after transplantation.

Fig. 3. The same: 10 days after transplantation. The homografts stand out more sharply against the granulations.

Fig. 4. Twenty-three days after the initial operation. The autograft pinches are united by a filmy sheet of epithelium, but only a few of the 1st-set homografts, near the margin of the grafted area, have coalesced. The 2nd-set homografts (H2) lie to the left of the autograft sheet.

Fig. 5. Thirty-one days after the initial operation. The autografts are further consolidated. The free-lying 1st-set homografts are represented only by milky white patches, but many have been overgrown by native epithelium growing in from the margin of the raw area (*o*). The coalesced island (*i*) may also be roofed by epithelium of native origin. The 2nd-set homografts are ragged and scrappy in appearance.

Fig. 6. Thirty-six days. Further ingrowth from the margins of the grafted area.

Plate 2

Fig. 7. Specimen A-5 (**D**). One large and several small multinucleate phagocytes creeping on to the external surface of the cuticle of a 5-day autograft at the point where it dips down into contact with naked granulation tissue. × 160, haematoxylin and eosin. (1.)

Fig. 8. Specimen A-10 (**A**). The growing edge of epithelium spreading from a 10-day autograft. Compare with fig. 9. Granular and corneal layers have not developed. The cytoplasm of the polymorphs which have invaded the epithelial layers is clearly distinguishable. ×160, haematoxylin and eosin. (10.)

Fig. 9. Specimen H-10 (**B**). Epithelium newly spread from a 10-day homograft: just behind the growing edge. The cells are flatter than those illustrated by fig. 8, but their cytological condition is the same. ×160, haematoxylin and eosin. (16·7.)

Fig. 10. Specimen H-10 (**G**). Orientated fibroblast reaction among the plasma cells in the bed of a 10-day homograft. ×160, van Gieson. (19·5.)

Fig. 11. Specimen H-10 (**E**). The epidermis of a 10-day homograft at the end of a cycle of desquamation. ×63, van Gieson. (19·6.)

Fig. 12. Specimen A-15 (**C**). A re-entrant wedge of epithelium in the inner ring of new outgrowth from a 15-day autograft. Heavy polymorph infiltration. ×160, haematoxylin and eosin. (23·4.)

Fig. 13. Specimen H-15 (**C**). 15-day homograft epithelium from the inner ring of new growth, freshly disengaged from the underlying granulation tissue. Cells swollen; some polymorph infiltration; staining reactions feeble, and other secondary pathological changes. ×160, haematoxylin and eosin. (28·4.)

Fig. 14. Specimen H-15 (**C**). Compare fig. 13: the same section, nearer the original 'pinch'. Pathological changes less pronounced. ×160, haematoxylin and eosin. (28·4.)

Fig. 15. Specimen H-15 (**G**). A fine tracery of new mature-staining collagen fibres underlying the dermis in the bed of a 15-day homograft. ×160, van Gieson (green filter). (31·4.)

PLATE 3

Fig. 16. Specimen A-23 (**AB**). The growing edge and outer growth ring of epithelium newly spread from a 23-day autograft. Polymorph infiltration; some empty cell spaces; no mature stratum granulosum and cuticle. ×160, haematoxylin and eosin. (34.)

Fig. 17. Specimen A-23 (**C**). Gross polymorph infiltration of a re-entrant column of epithelium from the inner ring of new growth round a 23-day autograft. ×160 haematoxylin and eosin. (34.)

Figs. 18, 19. Specimen H-23 (**E**). Dissolution of the epidermis over the roof of a 23-day homograft. The basal layer cells are breaking away and coming to lie free in a dermo-epidermal cavity. The majority of their nuclei have undergone lysis. Some wandering cells have invaded the cavity, but they are no more densely distributed there than in the collagen bundles of the dermis. ×160, haematoxylin and eosin. (37·4, 37·6.)

Figs. 20, 21. Specimen H2–8 (**E**). Compare with figs. 18 and 19: a similar process taking place in the roof of an 8-day 2nd-set homograft. Contrast with the epithelium over the roof of a 10-day 1st-set homograft (fig. 11). The nuclei have undergone complete lysis (fig. 20) or are sharply pyknotic (fig. 21). In this case there is a dense accumulation of pus in the dermo-epidermal cavity. A small number of these have invaded the epithelial layers, and the remains of their nuclei are visible in fig. 20. ×160, van Gieson. (46·3, 46·4.)

Fig. 22. Specimen H2–8. The outer surface of the dermis of the 8-day 2nd-set homograft illustrated in figs. 20 and 21. The collagen bundles have been infiltrated and prised apart by pus cells, but their specific staining power is not lost. ×160, van Gieson (green filter). (46·1.)

Fig. 23. Specimen H-36B. Fragments of cuticle imbedded in the granulation tissue round the dermis of a 36-day homograft, as a result of the process of overgrowth illustrated by a low-power photograph (fig. 26) of the same specimen. Vigorous giant-cell reaction. ×63, haematoxylin and eosin. (52·4.)

PLATE 4

Fig. 24. Specimen H-36A. Strips of cuticle far out over the granulation tissue surrounding a 36-day homograft: showing that outgrowth of epithelium had taken place. Giant-cell reaction. ×63, haematoxylin and eosin. (50·5.)

Fig. 25. Specimen H-36A (**E**). All that is left of the epidermis over the roof of the 36-day homograft illustrated by fig. 24: a pocket supported by cuticle and containing cellular debris. The nuclear spaces are empty. Multinucleate giant cells growing over the outer surface of the cuticle. ×63, haematoxylin and eosin. (49·2.)

Fig. 26. Specimen H-36B. Granulation tissue overgrowing and beginning to imbed the dermis of a 36-day homograft. Strands of cuticle have been enveloped, and these are invested by giant cells (fig. 23). A small fragment of epithelial cell debris is still distinguishable in the aperture left by the closing lips. ×16, haematoxylin and eosin. (52·6.)

Fig. 27. Specimen H2–17. The remains of a 17-day 2nd-set homograft. No trace of epithelium is present, and the collagen bundles of the dermis are highly eroded. ×16, van Gieson (green filter). (59·5.)

Fig. 28. Case F, specimen B. The remains of the collagen bundles of the dermis of a pinch homograft 2 months after transplantation. The dermis is now completely overgrown by granulation tissue in the manner foreshadowed by specimens H-36B (fig. 26) and H-36C. See fig. 33. ×16, van Gieson (green filter). (8·6.)

Figs. 29–32. Case F, specimen B. Phagocytosis of collagen by mononuclear phagocytes. Masson stain. Through a green filter, the green of the collagen and the red of the cytoplasm are transposed to whitish and dark shades respectively (though not in the very deeply staining fibre of fig. 32, which is still extracellular). Some of the enclosed fragments retain traces of a fibrous structure (fig. 31). ×375. (9·1, 9·2.)

Fig. 33. Case F, specimen B. Collagen 'erosion' (see fig. 28). The process described as erosion is one in which the collagen bundles splay out, lose their specific staining power, and merge gradually into the reticular ground-substance of the granulation tissue. ×160, van Gieson (green filter). (8·6.)

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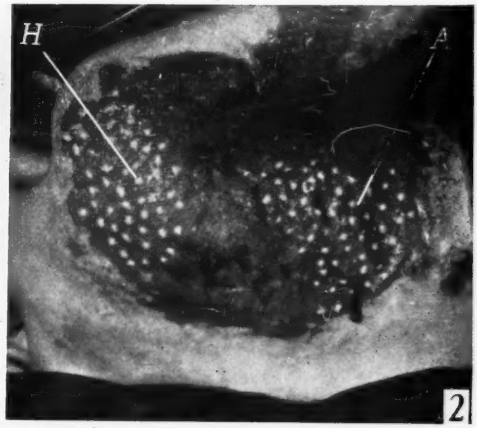
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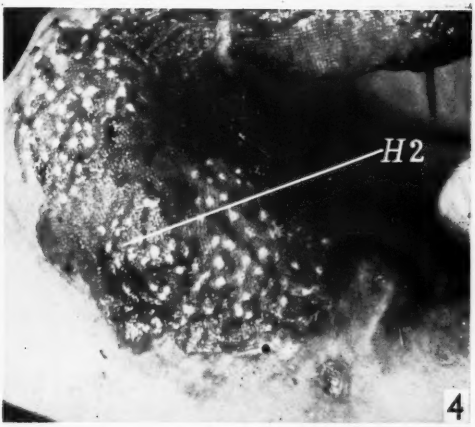
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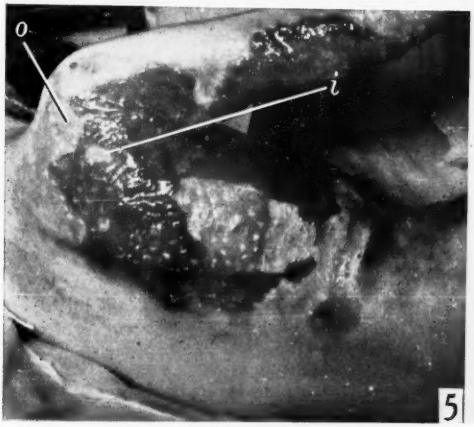
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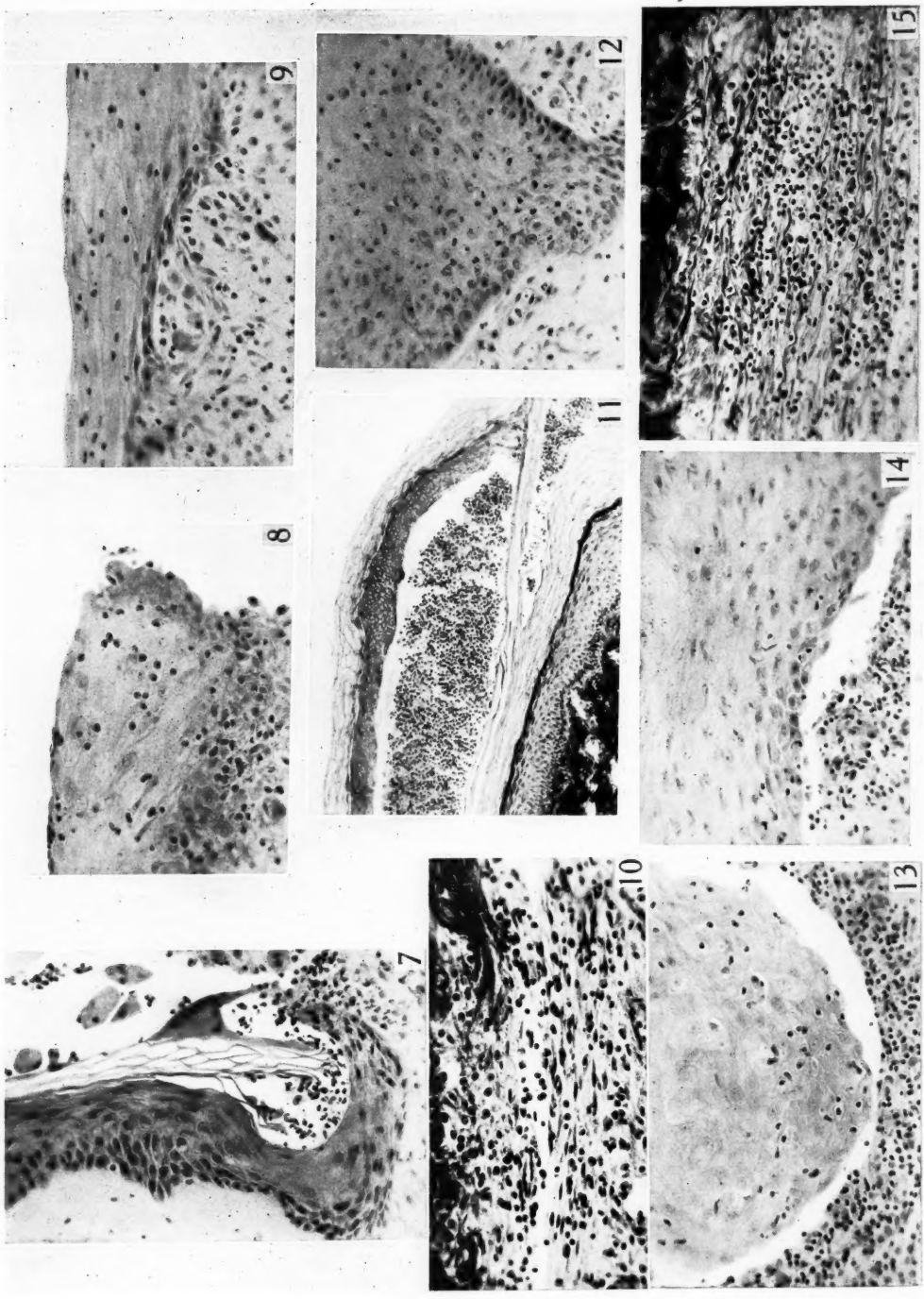
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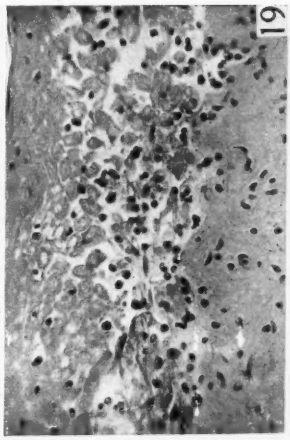
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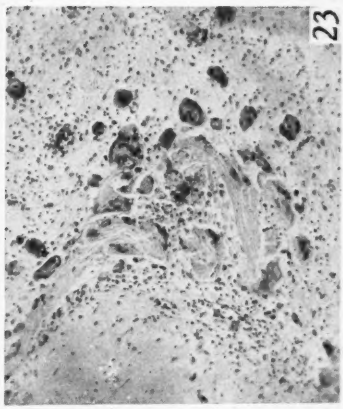
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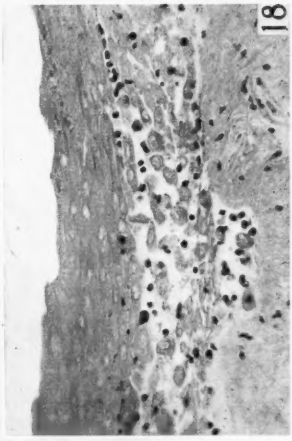
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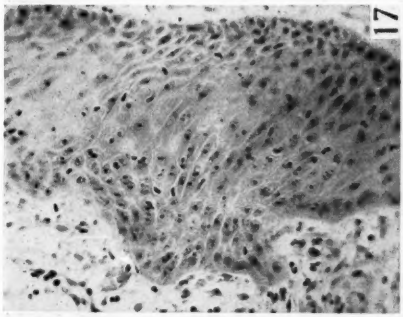
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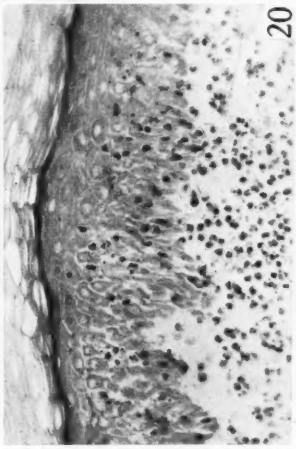
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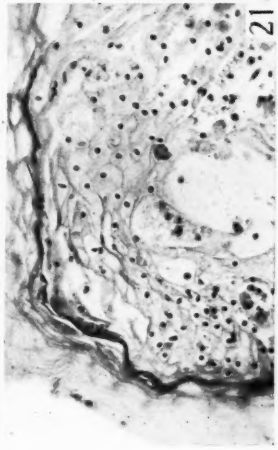
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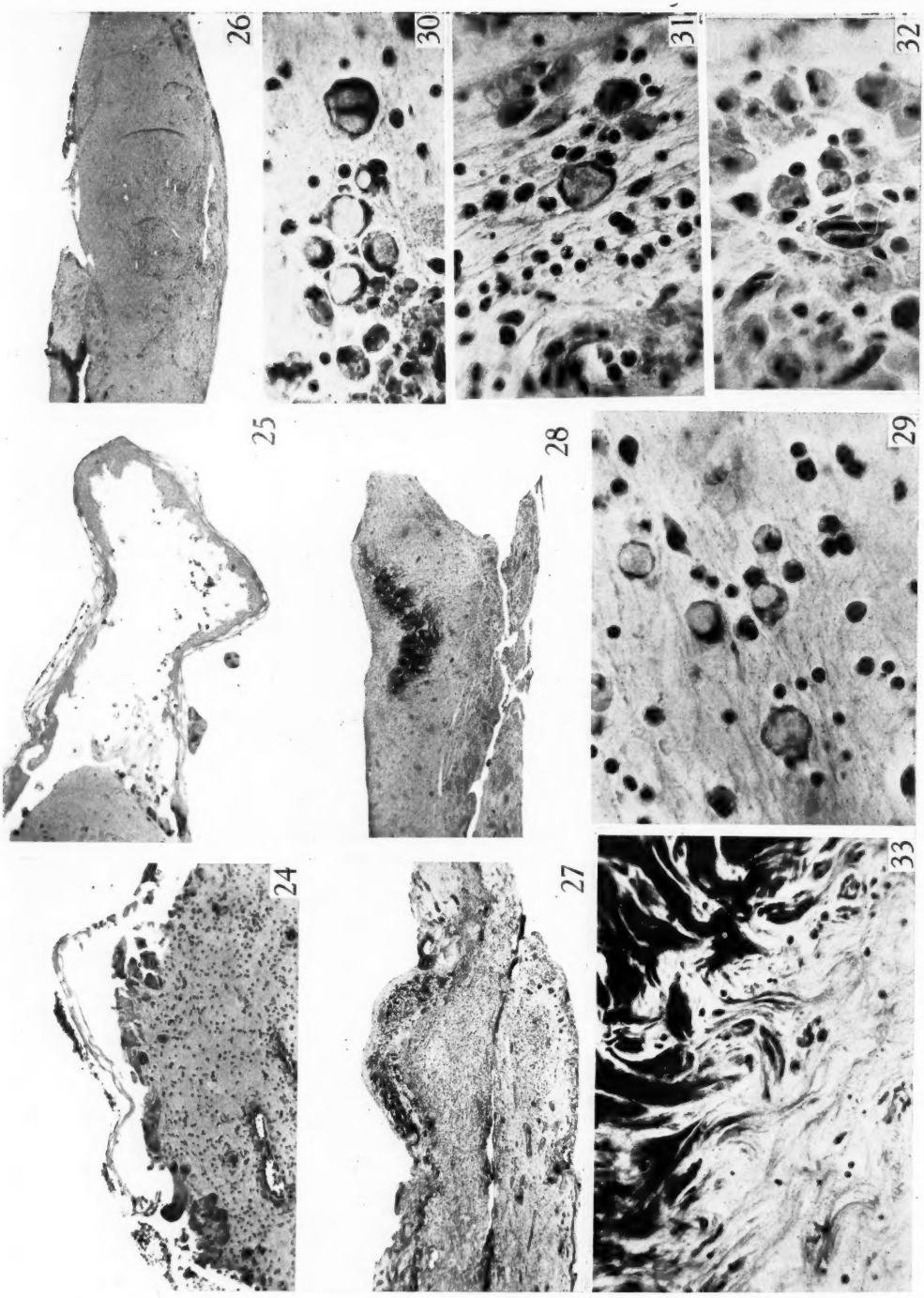

GIBSON AND MEDAWAR—SKIN HOMOGRAFTS

FREDERICK GYMER PARSONS

1863–1943

IN MEMORIAM

FREDERICK GYMER PARSONS, D.Sc., F.R.C.S., F.S.A., *Professor of Anatomy, University of London*

1863–1943

The death of Professor F. G. Parsons at the age of 80 marks the passing of one of the oldest representatives of British Anatomy. To many anatomists in the country it also marks the loss of a personal friend whose charm of manner was one of his most outstanding qualities. In the field of anatomy he was noted not only for many solid contributions to anatomical science but also for a great deal of laborious but unobtrusive work on behalf of the Anatomical Society and anatomical teaching. He had been a member of the Society since 1889, that is to say, two years after its foundation. Indeed, at the time of his death he was the oldest member of the Society with a membership record of 54 years. He also acted as its secretary from 1895 to 1898, its treasurer from 1903 to 1908, and its president from 1912 to 1914.

Parson's academic life was spent entirely at St Thomas's Hospital Medical School where he started his career as lecturer in biology, and he was lecturer and Professor of Anatomy there from 1886 to 1929. His earliest scientific work was in the field of comparative anatomy, and he first achieved distinction by his studies on comparative myology. Numerous papers on this subject dealing with the muscular anatomy of Carnivora, rodents, edentates, ungulates, etc. were published (partly in collaboration with B. C. A. Windle) in the *Proceedings of the Zoological Society*. Other important contributions on skeletal and joint morphology appeared during the same period in the *Journal of Anatomy*. The general conclusions which arose from these comparative studies were summarized in a series of outstanding Hunterian Lectures published in 1897 and 1899.

In his attitude towards this field of work, Parsons was a pure morphologist, for while he often sought to explain variations in homologous muscles in different mammals on the basis of functional requirements, the evolutionary implications attracted him most. He was, however, no wild theorist. Methodical and careful in his tedious work of manual dissection, he was also methodical and careful in his interpretation of the observations which he made. As a consequence, his work at this phase of his career is the more enduring, for his papers contain a vast amount of accurate and systematically recorded data which will always be available to comparative anatomists.

In the field of human anatomy Parsons was also a meticulous observer. He published a number of short papers dealing with the topography of soft tissues, among which may especially be mentioned his studies of the caecum, the parotid gland and the cervical fascia. The last named, which was illustrated by careful dissections, demonstrated the absurdities of some of the descriptions of fascial planes in current text-books and played no small part in leading to a much-needed revision.

In 1909 Parsons published a study of the topography and morphology of the human hyoid bone, an element of the human skeleton which hitherto had been curiously neglected in purely descriptive anatomy; thereafter, most of his anatomical papers were concerned with the skeleton. Of special note are his monographs on the characters of the English thigh bone and the proportions and characteristics of the English clavicle. These studies led Parsons from pure topographical anatomy to physical anthropology, and particularly craniology. He had devised a method for constructing average contours of the skull from different points of view, which provided an excellent means for visualizing the appearance of the average of a series of crania, and he used this technique in the systematic study of collections of skulls from Hythe and Rothwell. It has always seemed to me surprising that this method has not been more widely adopted, though a similar (but less effective) procedure was later advocated by Bennington and Pearson in *Biometrika*.

Later, Parsons made a systematic study of the skeletal remains of the early Anglo-Saxon population of England, and these records are particularly important as a basis of reference for future workers. In 1928 he published an atlas showing the contours of sixty-six Anglo-Saxon skulls which he had collected from various parts of the country. During the last war, Parsons seized the opportunity offered by prisoners of war to ascertain some objective facts about the racial constitution of the population of Germany, and he was able to show that, except in the coastal districts and along the valley of the Rhine, the so-called 'Nordic' element is very slight indeed.

Parsons's main interest in the physical anthropology of past populations of this country lay in its bearing on the constitution of the present population. In 1921 he published an important paper on the Long Barrow race and its relationship to the modern inhabitants of London, and he later developed his ideas in an unusually interesting book on *The Earlier Inhabitants of London*. In 1927 he was elected President of the Anthropology section

of the British Association which met at Leeds, and his presidential address was entitled 'The Englishman of the Future'. In this address he drew attention to certain changes in the physical proportions of the English population which seemed to him to be taking place. Among other things he concluded that the shape of the skull shows progressive changes particularly in the direction of an increase of the proportional height, and he adduced evidence to show that these changes must be regarded as part of a definite evolutionary process.

From a pure physical anthropologist Parsons in later years became more and more of an antiquarian. His life-long association with St Thomas's Hospital led him to make a special study of its history, and the results of his historical researches were published in an outstanding work of three volumes, which were completed in 1936. After his retirement he continued his antiquarian interests and published several short papers of archaeological interest in local journals.

To the teaching of anatomy he made many contributions. Among these may be specially mentioned his publication (in collaboration with William Wright) of a practical dissecting manual in 1912. The purpose of this book was to relieve the ordinary student of a great deal of cumbersome and unnecessary topographical detail and to focus his attention on the really important features of human anatomy. This book was regularly used in certain medical schools, but the time was not then ripe for it to displace the more elaborate manuals which it was commonly regarded as necessary that the student should wade through. The work which Parsons did for the Anatomical Committee of London in his position as Secretary over a period of many years will be remembered with gratitude by his colleagues, for during this time he did very excellent service for the maintenance of the supply of dissecting material.

In this short account I have endeavoured to give a general survey of the fine record of solid scientific work which Parsons has left behind him. A proportion only of his published works have been mentioned, and a reference to past numbers of the *Proceedings of the Zoological Society*, the *Journal of Anatomy* and the *Journal of the Royal Anthropological Institute* will show how productive he was in the fields of anatomy and physical anthropology. I should like now to give a more personal impression of this distinguished man. Parsons was essentially a craftsman. He loved working with his hands, and he was marvellously skilful with his scalpel and forceps. Many of his dissections will permanently adorn the museum of his own medical school, and some had the honour of being accepted to take their place among the exhibits in the Hunterian Museum of the Royal College of Surgeons. One of his hobbies was wood-carving. His own house was decorated with exquisite carvings of his own creation and he also applied his talent to his anatomical work in the construction of teaching models and in preparing models to show the average proportions of those elements of the English human skeleton which he studied. With his manipulative skill he always seemed to be far more interested in the actual preparation of material for his studies than in any scientific result which accrued from them. Indeed, he started each piece of work with no fixed preconceived ideas and was perfectly content to allow his data to speak for themselves. Thus he avoided one of the pitfalls likely to beset the comparative anatomist whose main object is to elucidate evolutionary processes, for he was careful to avoid phylogenetic speculations of wide implications. In the field of anthropology, also, Parsons seldom figured in the controversies which were apt to be rather common in his time, for his attitude to theoretical hypotheses was careful to the extent of being often rather colourless. I remember an occasion on which (with the late Professor Seligman) he was reading a paper on the palaeolithic skull from Cheddar before the Royal Anthropological Institute. In rising to reply to some criticism regarding the probable antiquity of the remains, Parsons made the remark 'I assure members of the Institute that my regard for this skull is purely platonic.' At a time when the echoes of the somewhat acrimonious controversy on the Piltdown skull had not yet died away, this remark naturally evoked a good deal of amusement. Yet it illustrated very well the attitude of detachment and objectivity which marked all his scientific work.

At one time he came in for a certain amount of criticism from the biometrical school on the ground that the mathematical treatment of his metrical observations was inadequate. However, he quite frankly disclaimed any particular aptitude as a biometrician and pointed out that the published data of his measurements were available to anyone who might wish to analyse them by the technique of the biometrical school. He was also somewhat scathing (and not altogether unjustly) of the type of mathematician who, without any biological training or insight, was wont to treat skeletal elements as so many geometrical figures without any reference to the functional factors which might play a part in determining their proportions.

At St Thomas's Hospital Medical School, Parsons was for many years the central figure, and many of the activities of the School were developed on his initiative and with his support. As friend and adviser his opinion was always being sought by his colleagues and pupils on all manner of problems, whether concerning administrative policy or purely personal affairs. He had an extraordinarily balanced outlook on life and he was gifted with an unusually acute sense of humour. These charac-

teristics enabled him to put many difficult situations in their proper perspective, both for himself and for others who sought his advice. His life was not without personal tragedy. The sudden death of his wife from an accident in 1915 was a grievous loss to him, and his friends knew well enough how deeply it affected him. Yet his philosophy of life endowed him with a resilience which enabled him to surmount the disaster and to carry on with his life's work with an amazing fortitude.

When he finally retired from St Thomas's Hospital, Parsons settled down in Princes Risborough. He had a house built in a beautiful situation on the slopes of the Chilterns. It was always a delight to visit him there and to stroll with him through the beautiful beech-woods of the Chilterns, for he always seemed happy and contented and his conversation was full of amusing reminiscences. He spent a great deal of time in his garden, and from time to time he continued actively to maintain his antiquarian interests by local archaeological studies. Indeed, with his garden, his country walks and his interest in local archaeology he seemed to revel in his retirement. Unfortunately, military exigencies compelled him to leave the home he had made, and soon after the beginning of the war he went to live in Thame. But, as always, he accepted the situation philosophically and found happiness in his new surroundings without any regrets. He lived there with his son and daughter-in-law.

With the final opportunity available to me before I was compelled under the restrictions of war to lay up my car, I paid a visit to Parsons at Thame in the summer of 1942. I am glad I did so, for this was the last time I saw my old teacher and friend. My outstanding memories of this visit are first that his vigour seemed unabated and his contentment with life as great as ever, and secondly, the great joy which he derived from the companionship of his little grandson.

W. E. LE G. C.

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